Applying nanopore sequencing for antimicrobial resistance gene analysis of bacteria isolated from patients.

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INTRODUCTION

At present, antimicrobial resistance is a growing concern with certain bacteria developing the ability to resist the effects of antibiotics. Antimicrobial resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019[1]. In Thailand, a strategic plan is in place to manage antimicrobial resistance, but a comprehensive strategy is still lacking. Globally, there is an increasing trend in antibiotic resistance surveillance, with a projected rise in mortality rates in the future.

Genes associated with antibiotic resistance often exist in the form of plasmids or transposons and can be transferred between cells through conjugation, transformation, or transduction. This horizontal transmission of resistance genes contributes to the rapid spread of resistance throughout bacterial populations and among different bacterial strains. This poses a significant challenge for healthcare professionals treating infectious diseases.

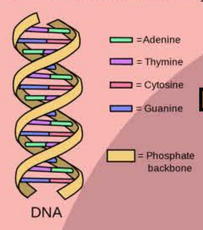
The Oxford Nanopore sequencing technology had gained popularity for real-time and long-read DNA sequencing, making it suitable for this application. Therefore, this project aims at employing nanopore sequencing, bioinformatics, and functional assay to identify the antibiotic resistance in bacteria isolated from patients and hospital environment.

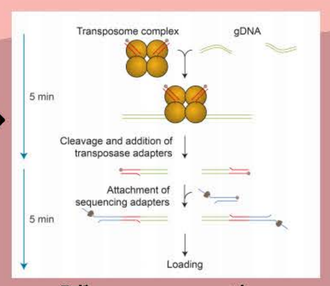
METHODS

Extraction of DNA from samples.

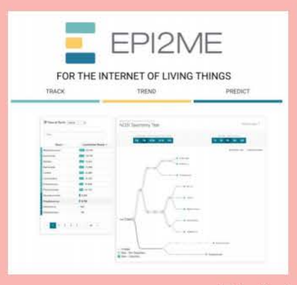


Bacteria isolated from patients.





Library preparation.



Bioinformatics analysis.





Oxford nanopore sequencing machine.

RESULTS & DISCUSSION (cont.)

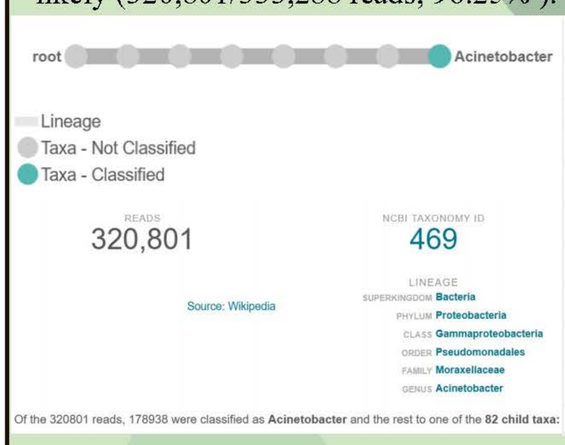
Table 1 Antibiotic gene alignments and top gene lists (> 100 alignments)

READS ANALYSED	ALIGNMENTS	AVERAGE ACCURACY	CARD GENES
333,288	4,471	84.2%	184

Gene	Alignments	Average Accuracy	Taxon	CARD Model
Salmonella enterica serovar Typhimurium 16S rRNA mutation in the rrsD gene conferring resistance to spectinomycin	276	77%	Salmonella enterica subsp. salamae	rRNA mutation model
adeG	241	84%	Acinetobacter baumannii SDF	protein homolog model
adeB	236	84%	Acinetobacter baumannii AB307-0294	protein homolog model
adeJ	235	86%	Acinetobacter baumannii	protein homolog model
ADC-2	156	84%	Proteobacteria	protein homolog model
LpxC	154	87%	Acinetobacter baumannii	protein variant model
abeM	151	85%	Acinetobacter baumannii	protein homolog model
mexT	141	85%	Acinetobacter baumannii SDF	protein homolog model
adeH	139	86%	Acinetobacter baumannii AYE	protein homolog model
adeK	135	86%	Acinetobacter baumannii	protein homolog model
LpxA	129	87%	Acinetobacter baumannii	protein variant model
msrE	119	85%	Escherichia coli	protein homolog model
TEM-4	108	86%	Klebsiella pneumoniae	protein homolog model
adeF	107	84%	Acinetobacter baumannii SDF	protein homolog model
adeI	107	85%	Acinetobacter baumannii AB0057	protein homolog model
adeA	106	85%	Acinetobacter baumannii AB307-0294	protein homolog model
Neisseria meningitidis 16S rRNA mutation conferring resistance to spectinomycin	104	77%	Neisseria meningitidis	rRNA mutation model
sul2	100	86%	Vibrio cholerae	protein homolog model

RESULTS & DISCUSSION

DNA library was prepared using Rapid Sequencing Kit V14 (SQK-RAD114, Oxford Nanopore Technologies). The sequencing and data retrieval was performed via MinION MK1C. The data analysis was performed via WIMP to identify the species and Fastq antimicrobial resistance alignment to identify the antibiotic resistant genes. The result of isolated clone identification was shown in **Figure 1** demonstrating *Acinetobacter baumannii* was most likely (320,801/333,288 reads, 96.25%).



Taxon	Reads
Acinetobacter baumannii	116,615
Acinetobacter calcoaceticus/baumannii complex	13,092
Acinetobacter nosocomialis	2,257
Acinetobacter seifertii	1,947
Acinetobacter pittii	1,494
Acinetobacter baumannii ACICU	600
Acinetobacter calcoaceticus	523
Acinetobacter baumannii ZW85-1	482
Acinetobacter baumannii ATCC 17978	433

Figure 1 Taxonomy classification of sample no. 1 using WIMP function of EPI2ME.

The sequence alignment via Fastq antimicrobial resistance showed the total reads been analized were 333,288 reads and 4,471 reads were successfully aligned with antibiotic resistant genes with 84.2 % of average accuracy. The antibiotic resistant genes (184 genes) were found to be involved in this clone. The data were analyzed through The Comprehensive Antibiotic Resistance Database[2]. The top gene lists (more than 100 reads aligned) were showed in **Table 1**.

NEXT PLAN

Process	Month											
	OCT	NOV	DEC	JAN	FEB	MAR	ARP	MAY	JUN	JUL	AUG	SEP
1. Literature review	■	■										
2. DNA extraction in sample		■	■									
3. Preparation library and analysis of sequencing data by Oxford nanopore sequencer.			■	■	■	■						
4. Bioinformatics analysis				■	■	■	■	■	■			
5. Manuscript preparation and submission										■	■	■

Acknowledgements

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660135]

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2. https://card.mcmaster.ca/

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PMU-B

Development of Machine Learning Tools for Braindead Disease Diagnosis Using MRI Spectroscopy

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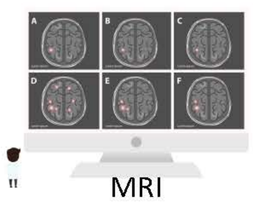
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BACKGROUND

Radiomics has gained significant attention in the field of neuroimaging, offering a non-invasive and quantitative approach to understand the underlying complexity of various neurological disorders[1-3]. While specific literature on radiomics for a condition, namely, "braindead disease" may be limited, studies on radiomics in neurological disorders can provide insights into the potential applications of this approach[1-4].



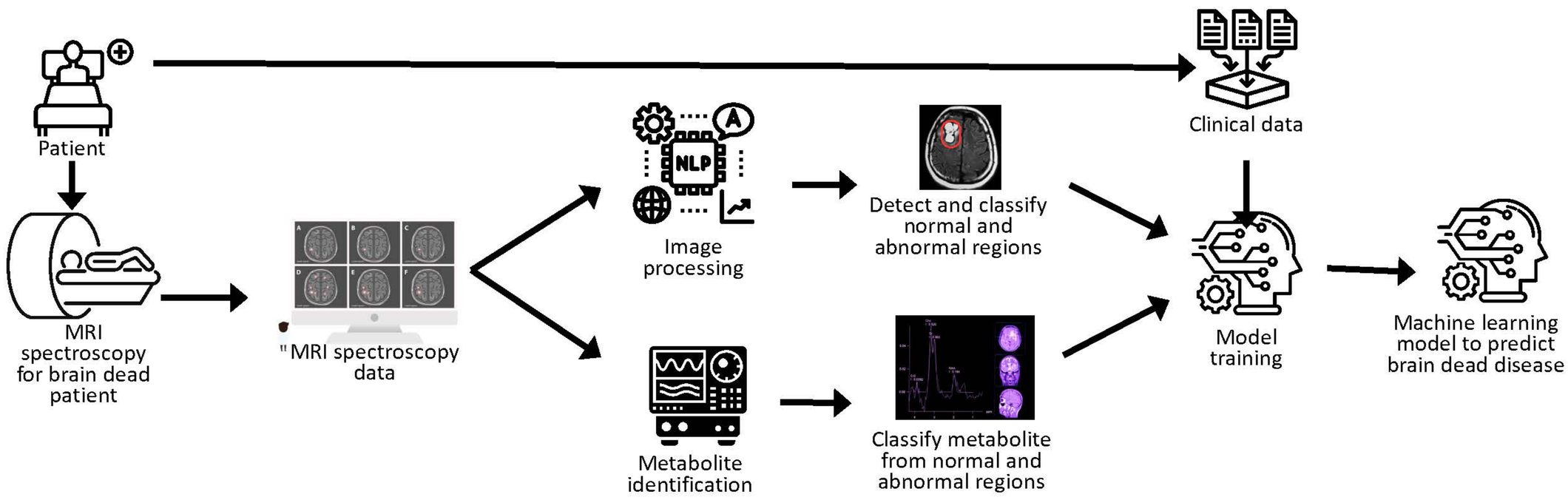
MRI spectroscopy data

The accurate diagnosis of brain diseases, particularly in distinguishing braindead regions from other conditions such as inflammation, remains a challenging task in clinical practice. Magnetic resonance imaging (MRI) is a powerful tool, but its sensitivity in specific cases can be enhanced through the incorporation of magnetic resonance spectroscopy (MRS). MRS provides valuable metabolic information that can aid in the differentiation of abnormal regions.

RESEARCH OBJECTIVES

- To develop machine learning models for MRI spectroscopy to distinguish braindead regions
- To integrate advanced algorithms for processing complex metabolic information
- To identify and validate metabolic biomarkers as indicators of braindead regions
- To validate models on diverse datasets for robustness and generalizability

CONCEPTUAL FREMEWORK



EXPECTED OUTCOMES

- Develop robust machine learning models for analyzing MRI spectroscopy data to differentiate braindead regions.
- Achieve a significant improvement in overall diagnostic accuracy, specifically distinguishing braindead regions from inflammation, enhancing MRI sensitivity.
- Identify and validate metabolic biomarkers indicative of braindead regions for informed diagnostic and treatment decisions.
- Validate and ensure the generalizability of developed machine learning tools across diverse datasets.

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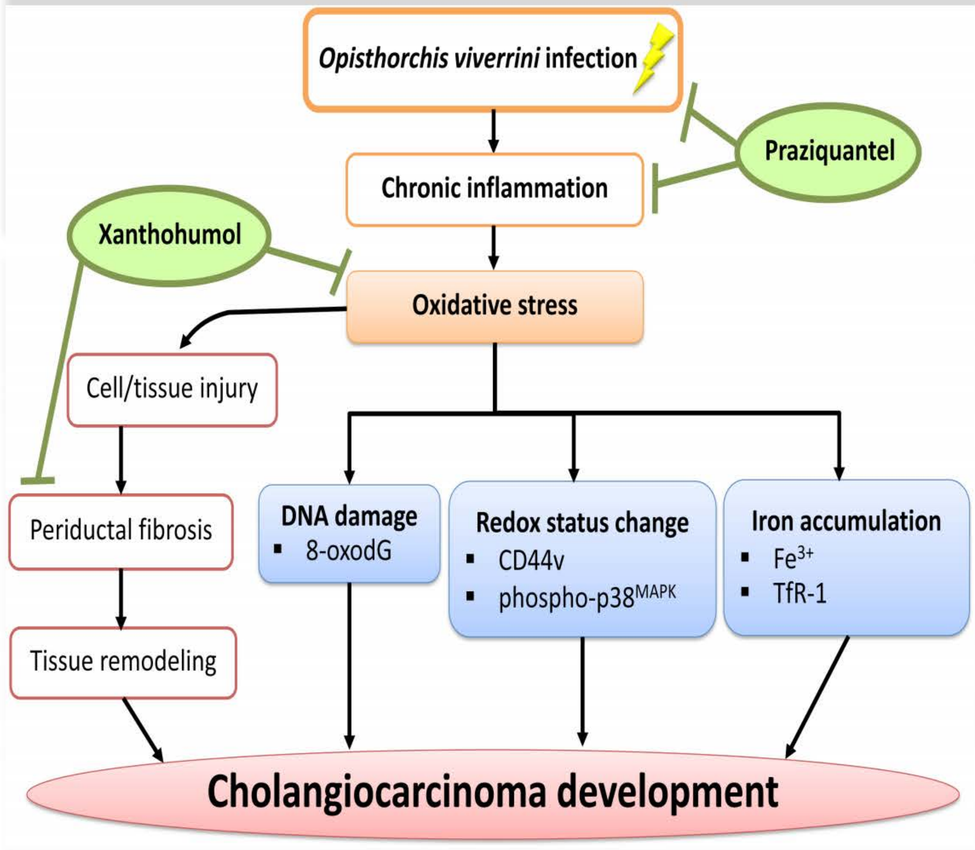
Urinary Biomarkers for *Opisthorchis viverrini* Infection

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INTRODUCTION

The liver fluke *Opisthorchis viverrini* (OV) is a fish borne trematode that causes hepatobiliary diseases and cholangiocarcinoma in the Lower Mekong River Basin especially in Thailand, Lao and People's Democratic Republic (Lao PDR). With no effective treatments for CCA currently available, early diagnosis and treatment of Ov infection is the most practical option for the prevention of CCA. Currently, biomarkers for OV infection and curative treatment relied conventional markers such as fecal egg, parasite-specific antibody and antigen in clinical samples. Previous studies suggested that DNA-adduct biomarkers in urine (8-oxodG) is elevated during opisthorchiasis and declined after drug treatment in some individuals (1). Recent studies using proteomic and metabolomic approach revealed many biomarkers for OV and CCA (2,3). These observations indicated the need for a more comprehensive study on biomarkers related to opisthorchiasis and the consequence of drug treatment.

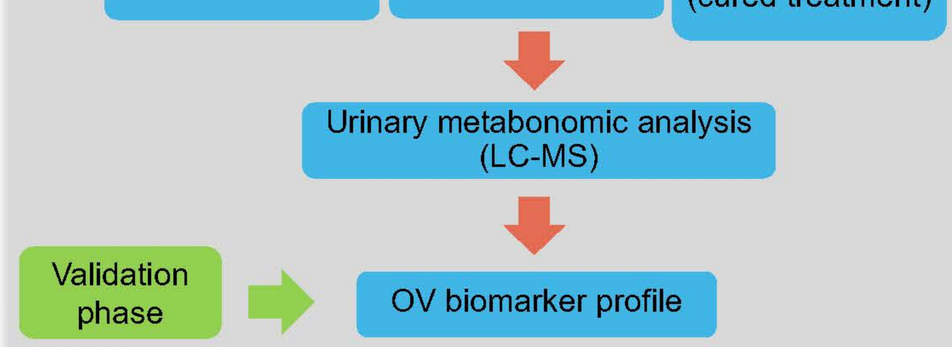


Roles of oxidative DNA damage on CCA – a potential inflammatory biomarker associated with opisthorchiasis (Jamnonkan et al, 2018) (4)

OBJECTIVE

The overall aim was to identify potential biomarkers for current and curative treated of opisthorchiasis in urine sample using a comprehensive metabolomic analysis.

METHOD



ANTICIPATE OUTCOME

The outcome of this study will provide a better understanding of the biomarkers relevant to current OV infection and those cured from drug treatment. The potential biomarker data are vital for the design of validation study in another set of population sample. These biomarkers provide a basis in a further study in morbility screening of patients with and without sign and symtoms of opithorchiasis including periductal fibrosis by ultrasonography. The potential biomarkers in urine in various clinical settings would provide more understanding of chronic opisthorchiasis and the impact of drug treatment which should reduce the risk of CCA.

ACKNOWLEDGEMENT

This research has receive funding support form the NSRF via the program management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660135]

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Effect of Melatonin and derivatives on wound healing and ex vivo skin permeation

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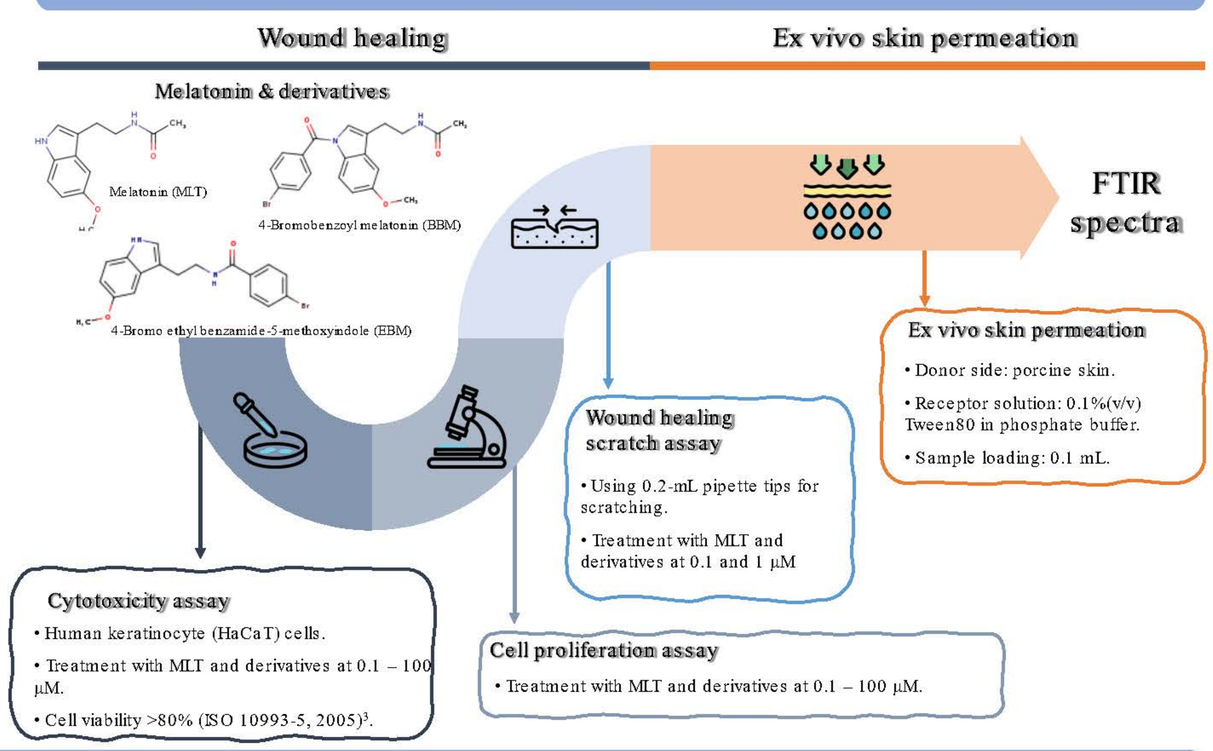
Introduction

Melatonin (MLT) is a renowned molecule which plays a wide variety of biological activities such as antioxidant, anti-inflammatory and wound healing properties.

4-Bromobenzoyl melatonin (BBM) and 4-Bromo ethyl benzamide-5-methoxyindole (EBM); MLT derivatives, were synthesized by our group by added substitution that affected anti-inflammatory and cell proliferation properties[1,2].

As its various properties of MLT derivatives, the skin cell proliferation and cell scratch assays were investigated to determine the role of MLT derivatives during wound healing.

Methods



Results

Wound healing study

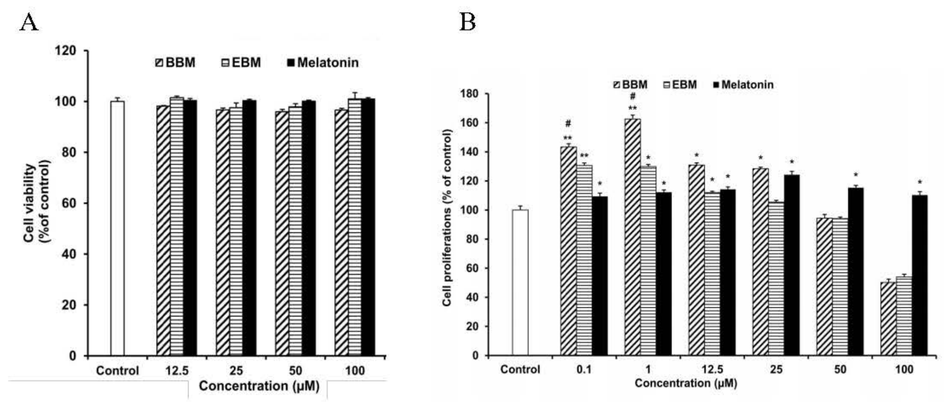


Figure 1. Effect of BBM, EBM, and MLT on HaCaT cells. (A) Effect of MLT and derivatives on cell viability (B) Effect of MLT and derivatives on cell proliferation.

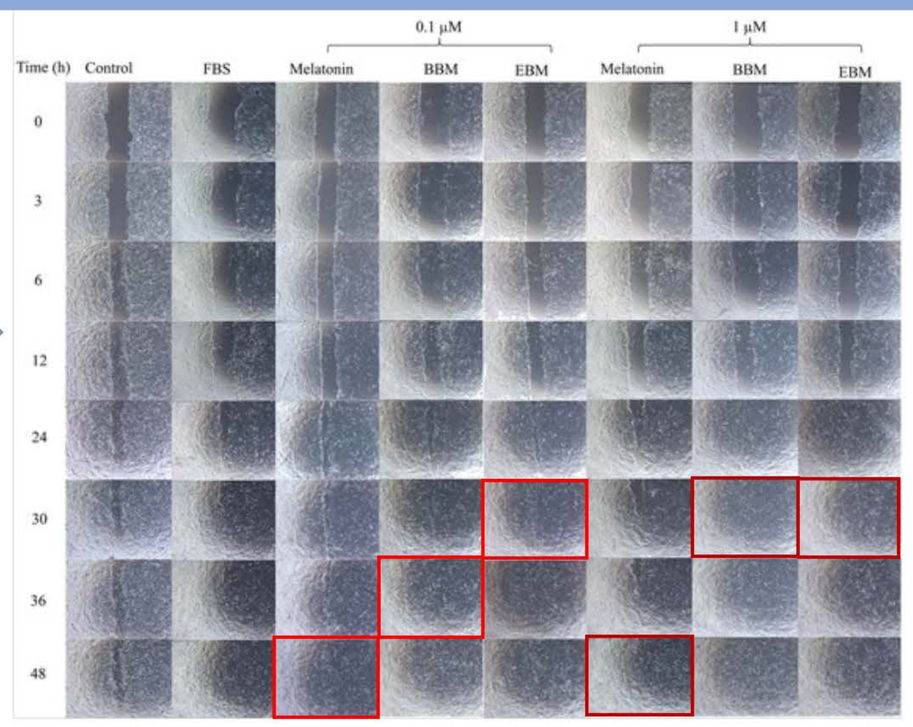


Figure 2. Effect of BBM, EBM, and MLT on wound closure of HaCaT cells after treatment for 48 hr. The wound margin was photographed at different treatment time. Red box in the image marks the percentage of wound closer >99%

- BBM and EBM could promote cell proliferation and cell migration on wound area better than MLT.
- Scratch wound healing assay: BBM, EBM >> MLT

Skin permeation study

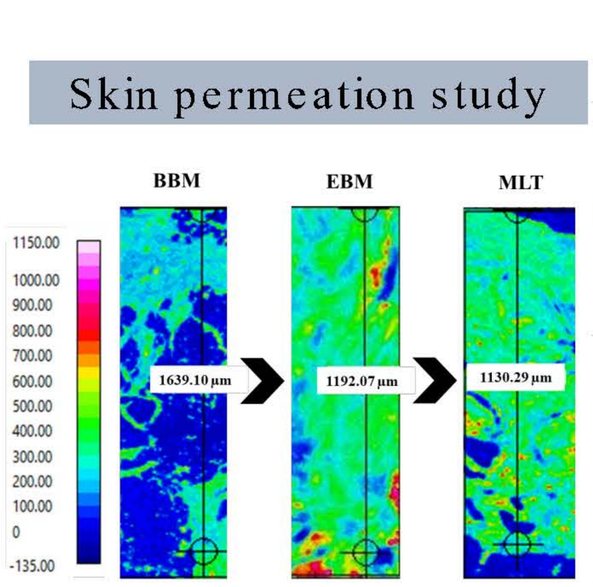
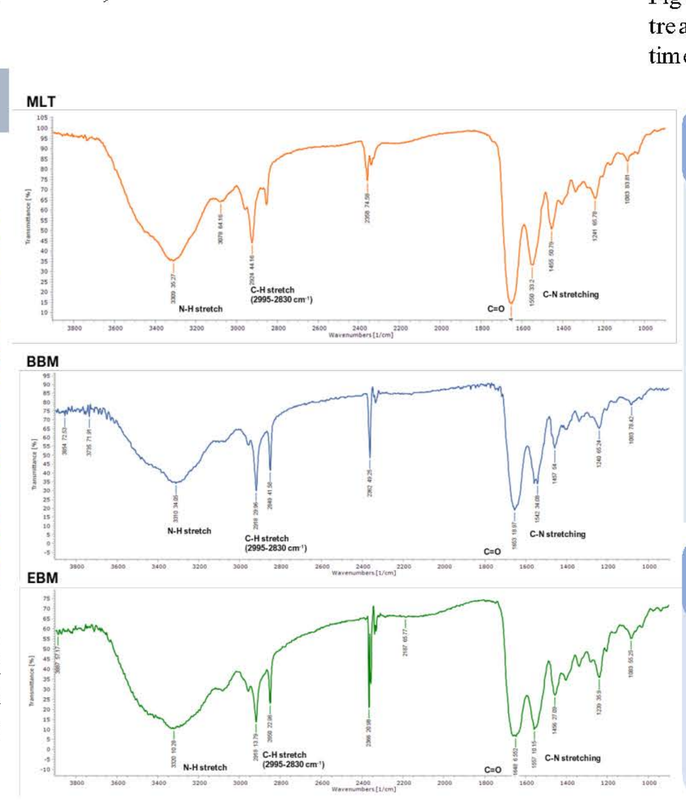


Figure 3. FTIR image with a distance from dermis to epidermis layers in the image (Left) and extracted spectrum (Right) from a cryo-sectioned porcine skin treated with melatonin and derivatives (BBM and EBM).



Conclusion

- Both molecules have a potential of wound healing activity greater than melatonin.
- Melatonin derivatives (BBM and EBM) promoted cell migration on wound area to 99% at 30 hr.
- FTIR spectra confirmed that BBM and EBM could permeate from epidermis to dermis layers faster than melatonin.

Acknowledgements

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660136].

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Effect of preparation methods for lycopene from vegetable extracts - loaded in niosome for topical applications

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Abstract

Lycopene niosome formulations from tomatoes, carrots, and red vegetables made using thin film and microfluidic method incorporated into preparations were developed for topical applications. Lycopene is an antioxidant, offers protection against ultraviolet (UV) radiation and exhibits tyrosinase activities. The physical properties of niosome formulations from these two methods were determined. The important parameters include particle size, polydispersity index (PDI), Zeta potential, and entrapment efficiency. The lycopene-niosome formulations from the microfluidic method provided better uniformity, homogeneity (low PDI value), and direct control of smaller sizes through a chip using a hydrodynamic flow focusing (HFF) platform than a conventional (thin-film) method. Lycopene-niosome formulations display sustained control of lycopene release. Additionally, All formulations showed morphology that presented vesicles found in a spherical unilamellar structure with a homogenous particle size and a smooth boundary. Therefore, lycopene niosomes from these extracts using microfluidic method can be used in place of those made using a thin-film method. The microfluidic method requires fewer process steps, which is beneficial in developing industrial niosome production processes to produce material for topical applications.

Keywords: lycopene-niosome formulations, thin-film method, microfluidic method, topical applications

Introduction



Lycopene is an important carotenoid pigments. It is a red pigment of vegetables and fruits with health benefits (Pk and P, 2016) . Lycopene has a non-conjugated double bond structure and 11 conjugated acyclic structures, which gives it strong antioxidant properties (Palozza et al., 2011). Tomatoes, carrots, and red vegetables are abundant natural lycopene sources that are used to develop health product supplements (Bin-Jumah et al., 2022)



Niosomes are bioactive compound delivery systems comprised of a non-ionic surfactant that forms a bilayer vesicular structure that can entrap bioactive compounds. The cholesterol used to make niosomes possesses both lipophilic and hydrophilic properties in creating its unique membrane structure.

Microfluidic methods are another newly developed technique used to prepare niosomes. Microfluidics produce materials for vesicular drug delivery using a chip device specifically designed to control the particle size of niosomes for drug encapsulation. Niosome formulations can be controlled during the mixing of two or more inlet streams that consist of lipids dissolved in an organic solvent and an aqueous phase. This is done using microfluidic equipment to control the flow rates of the organic and aqueous phases during mixing to achieve a specific niosome formulation.

Objective

This study aims to quantitatively investigate and characterize lycopenes according to their vegetable sources, biological activities, and develop lycopene-niosome preparation methods. Tomatoes, carrots, and red vegetable extracts subjected to a microfluidic method were compared with a thin-film method through physical characterization.

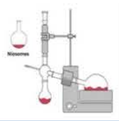
Methods

Niosome formulations



- Tomato, Carrot, and Red vegetables powder
- Microfluidic, and Thin film method
- The physical characterization

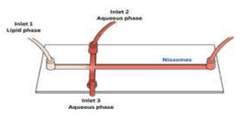
Thin film hydration method



- The preparation of non-ionic surfactant **(Span 60)** , and **cholesterol**
  - With 1:1 mM ratio for an organic phase
  - The organic phase was evaporated in a rotary evaporated to produce a thin – film.
  - The film on the rotating flask inner wall was dried for 30 minutes
- The preparation of tomato **(E2)**, carrot **(E3)**, and red vegetables **(E4)** extracts
  - With hydration technique
  - The niosome was reduced size by sonication probe (Amplitude 40%, 50 sec sonication with a 10 sec pause
  - The film on the rotating flask inner wall was dried for 30 minutes

Microfluidic method

- The preparation of non-ionic surfactant **(Span 60)** , and **cholesterol**
  - With 1:1 mM ratio for an organic phase
- The preparation of tomato, carrot, and red vegetables extracts
  - With aqueous phase
- Organic phase was mixed Aqueous phase in microfluidic mixing with a lab – on-a-chip by hydrodynamic flow focusing platform



The physical characterization → Entrapment efficiency, Zeta – potential, Particle size, and PDI → Releasing properties → TEM properties

Results and Discussions

Table 1. Entrapment efficiency (%), Zeta-potential (mV), Particle size (nm), and PDI values of niosome formulations made by thin-film hydration (T) and microfluidics methods (MF)

Method	Formulation	EE (%)	Zeta-potential (mV)	Size (nm)	PDI
Thin-film hydration (T)	E1	0.00 ± 0.00	-44.80 ± 1.63	245.16 ± 43.97	0.54 ± 0.008
	E2	98.16 ± 2.84	-36.76 ± 1.96	502.36 ± 33.52	0.85 ± 0.11
	E3	99.46 ± 0.11	-33.13 ± 2.75	429.30 ± 66.00	0.68 ± 0.68
	E4	95.04 ± 8.67	-31.56 ± 0.25	401.66 ± 61.01	0.72 ± 0.10
Microfluidic (MF)	E1	0.00 ± 0.00	-61.50 ± 0.90	257.46 ± 33.23	0.72 ± 0.28*
	E2	92.67 ± 0.15	-48.76 ± 3.54	252.10 ± 45.34*	0.41 ± 0.05*
	E3	95.82 ± 1.25	-42.53 ± 1.19	248.26 ± 39.51*	0.57 ± 0.16*
	E4	89.78 ± 0.54	-42.33 ± 1.10	252.10 ± 45.34*	0.68 ± 0.10*

Data represent the mean ± SEM values of three replicates. Statistical significance was evaluated by Dunnett post-hoc test (* $p<0.05$ compared with the thin-film hydration method).

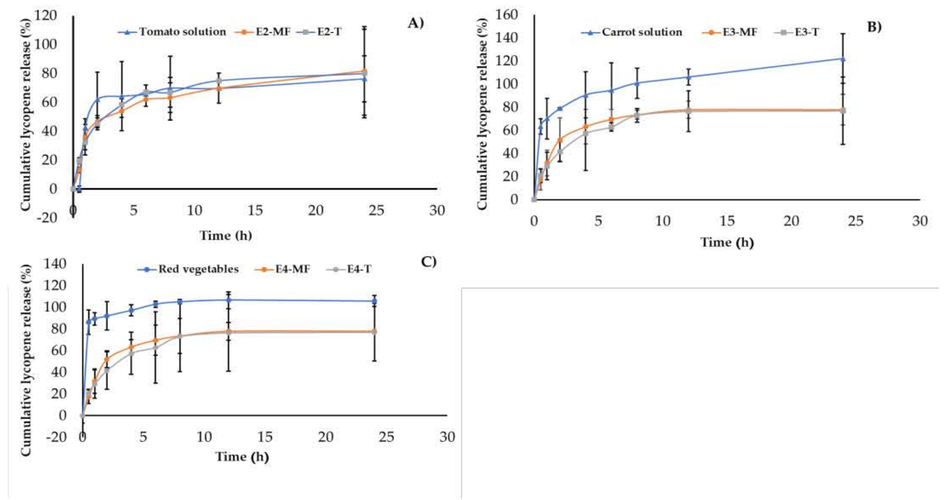


Figure 1. Release profiles of lycopene solutions from tomato, carrot, and red vegetable powders compared with niosome formulation using both methods, A) Lycopene extract from tomatoes, and E2 formulations, B) Lycopene extract from carrots, and E3 formulations, C) Lycopene extract from red vegetable powders, and E4 formulations.

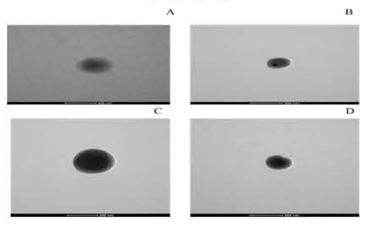


Figure 2. The morphology of MF-niosomes imaged using transmission electron microscopy (TEM) A) Blank niosome, B) E2 formulation, C) E3 formulation, D) E4 formulation (magnification ×25,000).

Conclusion

We show the effects of various niosome formulations with lycopene extracts from tomatoes, carrots and red vegetables. Lycopene from these extracts by the microfluidic method should be used in place of those from the thin-film method. Since the fewer steps are required, making the former process more suitable for industrial niosome production. Moreover, this technique also has greater uniformity, homogeneity (low PDI value), and precise management of niosomes with a smaller size. Additionally, all lycopene from these extracts provided sustained release. Lycopene-niosome formulations were applied as a topical product.

Acknowledgements

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Garcinia nigrolineata resin extract on Antioxidant activities, MAO inhibition and Depressive-like Behaviors Induced by CMS Mice Model

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Suphawut Chulikhit[5] and Orawan Monthakantirat[6,*]

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Introduction

Major depressive disorder (MDD), which is characterized by clinical symptoms such as sadness, depressed mood, anhedonia, hopelessness, and suicidal tendencies, is emerging as a major contributor to the global burden of disease[1]

The chronic mild stress (CMS) model of depression is the best described and most thoroughly validated animal model for depression. The CMS is the most acceptable model for screening antidepressants in animal models and supported understanding the neurobiological mechanism of stress-related disorder[2]

Garcinia nigrolineata (GN) Planch. ex T. Anderson is a small to medium-sized tree that is found throughout Southeast Asia[4]. A prenylated xanthone from resin attenuation of β-amyloid-oligomer inhibiting amyloid aggregation, we also investigated the neuropharmacological effect of the GN resin extract in the chronic mild stress (CMS) mice model and evidence suggests that all the chemical ingredients in the GN have antioxidant, neuroprotective, and antidepressant-like properties.



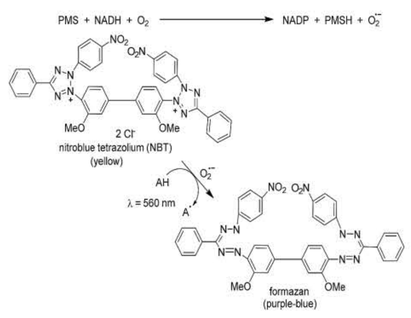
Methodology

1. In vitro studies

Antioxidants studies
- (1) Superoxide radical scavenging activity assay
- (2) Hydrogen peroxide scavenging activity assay

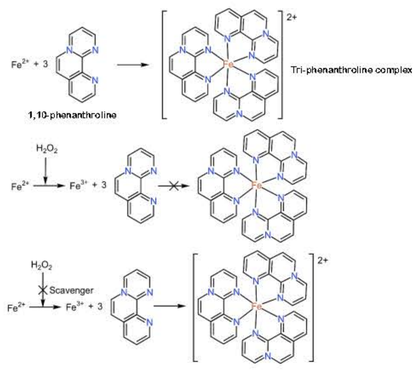
(1)





(2)





MAO Inhibitory effect
- (1) MAO- A inhibitory
- (2) MAO-B inhibitory

MAO-A



Sample	20 µl
Kynuramine	9 µl
Buffer	469.5 µl
MAO-A	1.5 µl

MAO-B

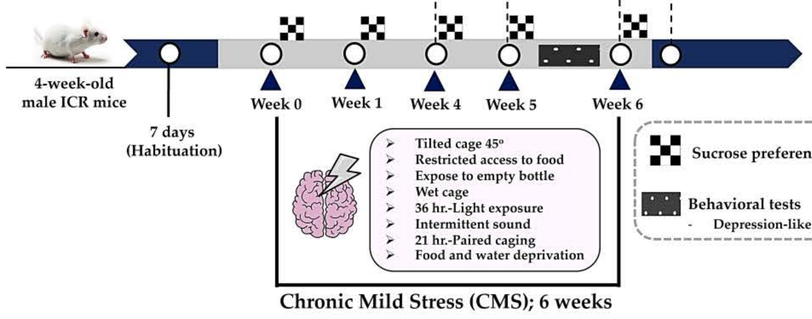


Sample	20 µl
Kynuramine	6 µl
Buffer	472.5 µl
MAO-B	1.5 µl

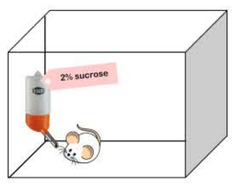
Incubate at 37 ºC 20 minutes → Add 400 µl of NaOH 1,000 µl of water → Measure at Excitation: 310 nm Emission: 400 nm → Calculate for % inhibition & IC_{50}

2. In vivo study

1. Control: 0.5% SCMC (Non-stress), (0.2 mL/40g; p.o.)
2. CMS + Vehicle: 0.5 g SCMC in 100 mL (0.2 mL/40g; p.o.)
3. CMS + IMP 20: 40 mg in 10 mL (0.2 mL/40g; i.p.)
4. CMS + GN 50 : 100 mg in 10 mL (0.2 mL/40g; p.o.)
5. CMS + GN 150: 300 mg in 10 mL (0.2 mL/40g; p.o.)
6. CMS + GN 450: 700 mg in 10 mL (0.2 mL/40g; p.o.)



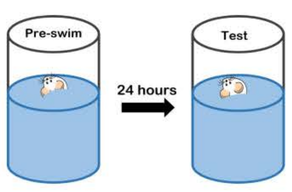
3. Behavioral study



Sucrose preferences test



Tail suspension test



Forced swimming test

Results

1. In vitro Results

Table 1. IC_{50} of G. nigrolineata resin extract for antioxidant systems.

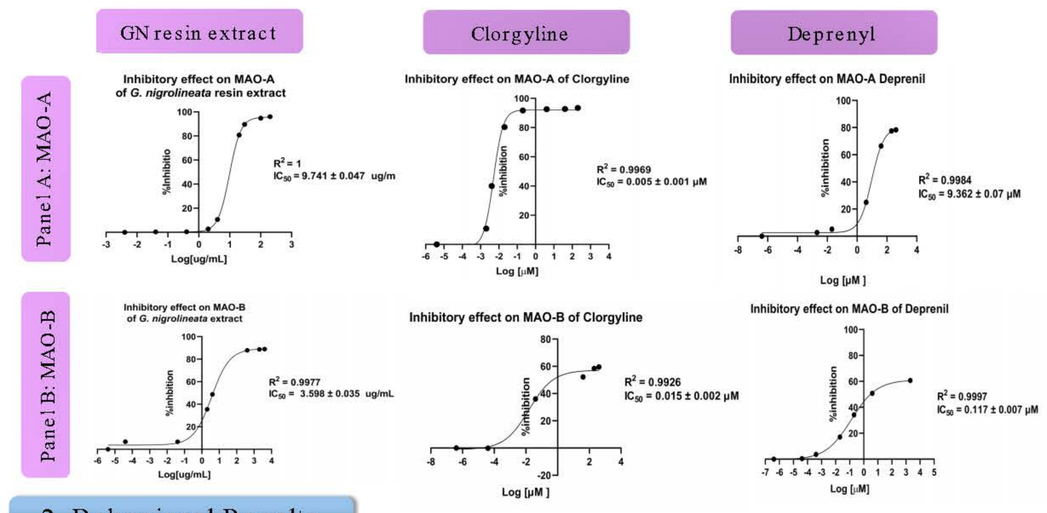
Sample / Reference	Scavenging activity assay	
	Superoxide radical IC_{50} (ug/ml)	Hydrogen peroxide IC_{50} (ug/ml)
G. nigrolineata resin extract	345.1 ± 6.3	152.8 ± 2.1
Trolox (µM)	81.85 ± 0.89	237.25 ± 2.62

Each value in the table is represented as Mean ± SD (n = 3).

Table 2. Inhibitory effect of G. nigrolineata resin extract, Clorgyline, and Deprenyl on MAOI.

Extract/ Compound	IC_{50} (µg/mL)		Ki (µg/mL)		Si		Selectivity
	MAO-A	MAO-B	MAO-A	MAO-B	MAO-A	MAO-B	
GN resin extract	9.741 ± 0.047	3.598 ± 0.0353	2.321	1.550	1.665	0.668	B
Clorgyline [1]	0.005 ± 0.001 µM	0.015 ± 0.002 µM	0.001	0.07	0.143	7	A
Deprenyl [2]	9.362 ± 0.07 µM	0.117 ± 0.007 µM	2.234	0.046	48.565	0.021	B

[1] Clorgyline is a selective MAO-A inhibitor, [2] Deprenyl is a selective MAO-B inhibitor, Si = Selectivity index.



2. Behavioral Results

Sucrose preference test

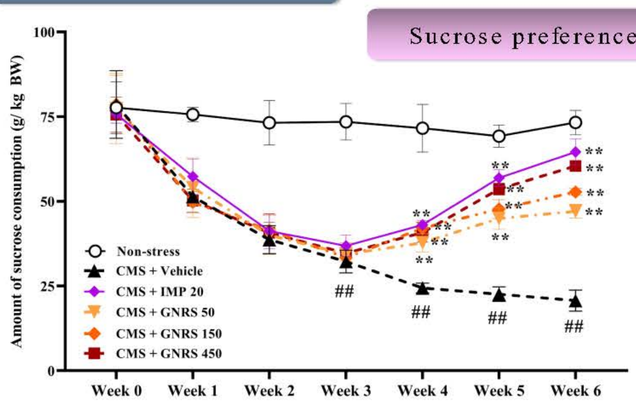


Figure 1. The effect of the GN resin extract on CMS reduced anhedonia behavior on the sucrose preference test. The amount of 2% sucrose solution taken by each animal group was measured as an index of sucrose preference test once a week. Each data point represents the mean ± SEM (n=12). ## p < 0.001 vs. the vehicle-treated non-stress group. ** p < 0.001 vs. the vehicle-treated CMS group.

Tail suspension test (TST)

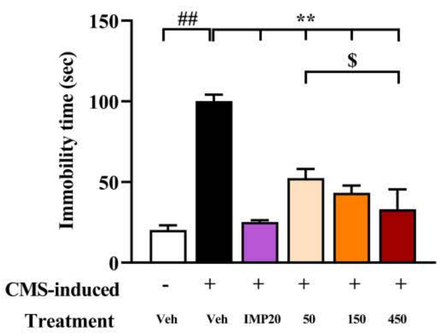


Figure 2. The effect of the GN resin extract on CMS-induced hopeless behaviors can produce a change in the behavior in the depression test model in TST. Each column illustrates the mean ± SEM (n=12). ## p < 0.001 vs. the vehicle-treated non-stress group. ** p < 0.001 vs. the vehicle-treated CMS group , and $^{\$}$p< 0.05 compared between doses of 50 and 450 GN resin extract.

Forced swimming test (FST)

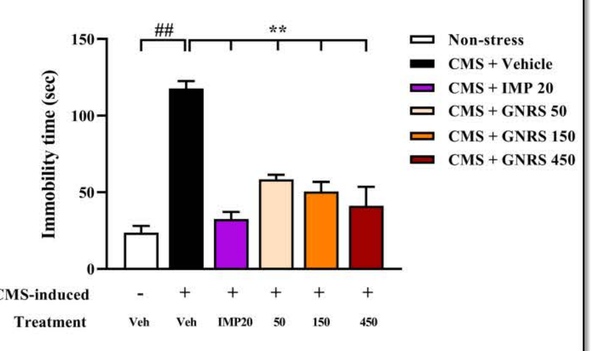


Figure 3. The effect of the GN resin extract on CMS-induced hopeless behaviors can produce a change in the behavior in the depression test model in FST. Each column illustrates the mean ± SEM (n=12). ## p < 0.001 vs. the vehicle-treated non-stress group. ** p < 0.001 vs. the vehicle-treated CMS group.

Discussion & Conclusion



In the present study, we found that CMS significantly increased anhedonia and depression-like behavior when compared with the vehicle-treated non-stress group. Mice treated with GN resin extract showed a significantly decreasing anhedonia-like behavior on sucrose preference and depression-like behaviors on TST and FST as same as imipramine. This enhancement could be reversed by long-term treatment of GN resin extract as well as by imipramine. Further studies are needed to clarify this possible mechanism by assessment of related gene expression on RT-PCR.

Acknowledgement

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660136] and Faculty of Pharmaceutical Sciences, Khon Kaen University including Private companies participate research under the Banya Group.

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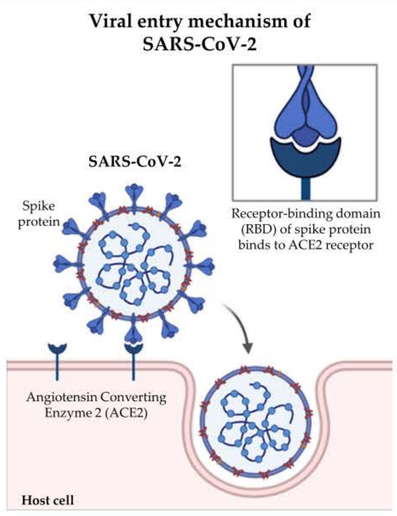
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Stability Evaluation of Mutant Angiotensin Converting Enzyme 2 (ACE2) decoy Produced in Plants

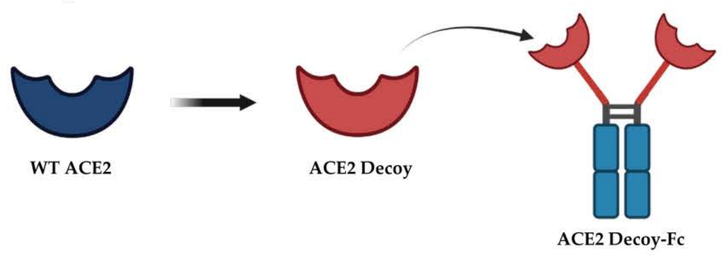
Chalisa Panapitakkul[1], Panithi Saktrakulkla[1]

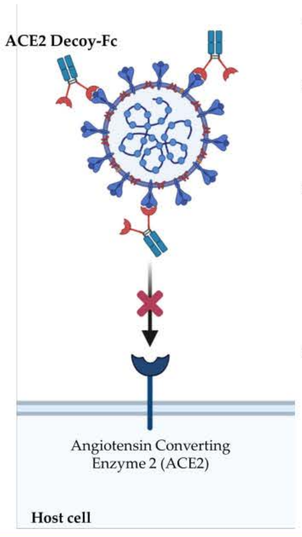
[1]Baiya Phytopharm Co., Ltd, Thailand

Introduction



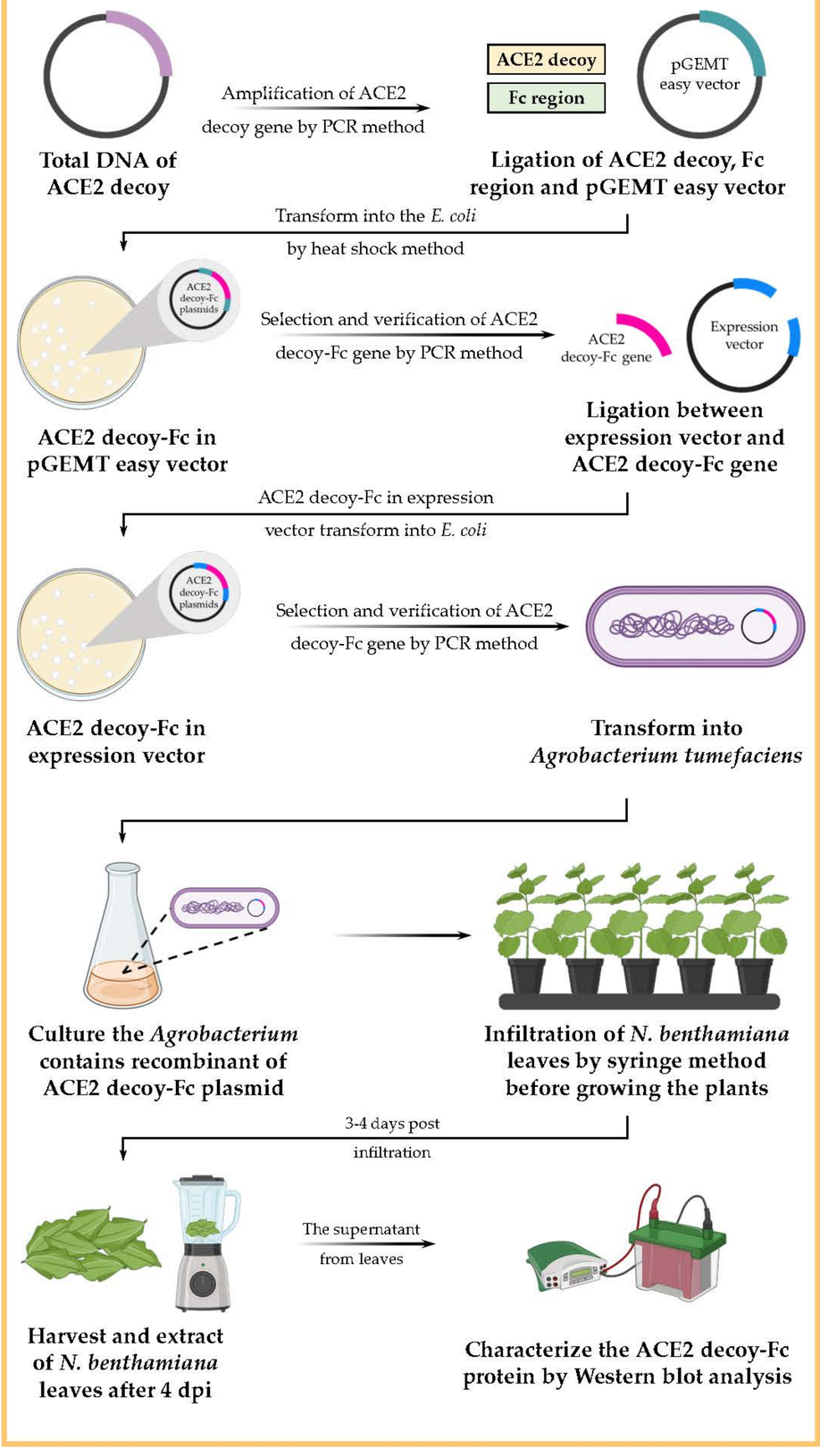
- ACE2 receptor is a necessary factor that helps SARS-CoV-2 infection
- ACE2 receptor can bind spike receptor binding domain (S-RBD)
- The engineered ACE2 containing mutations enhance affinity toward SARS-CoV-2 spike protein





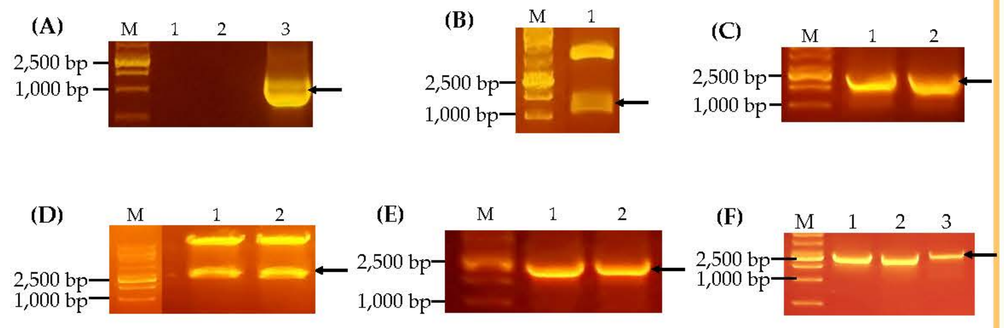
- ACE2 decoy can competes with endogenous ACE2 to bind S-RBD
- It can inhibit infection may offer a therapeutic option with sustained efficacy against SARS-CoV-2
- Therefore, we aim to identify suitable excipients in order to ensure the stability of this recombinant ACE2 decoy for long term
- These studies will help to identify the ideal formulation to take forward this plant produced ACE2 protein for further testing in animal models.

Method



Results & Discussion

- **Cloning ACE2 decoy-Fc gene and *Agrobacterium* transformation**



- The results showed that the ACE2 decoy gene can clone by PCR method **(A and B)** and ligate with Fc region into pGEMT easy vector **(C and D)** which can use this gene to ligate into expression vector **(E)** after that transform into *Agrobacterium* to use this plasmid to infiltrate in the plants **(F)**

- **Expression of ACE2 decoy-Fc protein**



- The results showed that the ACE2 decoy-Fc can be expressed in *N. benthamiana* plants with high yield obtained within 4 days after infiltration
- The characterization of plant-produced ACE2 decoy-Fc protein indicated that it has the size of approximately 100 kDa in reducing conditions **(G)**
- The result showed that the some of the proteins could be aggregated inside the plant cells

Conclusion

- **Cloning ACE2 decoy-Fc gene and *Agrobacterium* transformation:** can clone ACE2 decoy gene and use this gene to ligate with the Fc region into expression vector then use this vector to transform into *Agrobacterium* before infiltrating into the plants
- **Expression of ACE2 decoy-Fc protein:** after infiltration, the plants can produce the ACE2 decoy-Fc protein that express on the leaves

Acknowledgement

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] and Baiya Phytopharm Co., Ltd. Thailand

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Development of Nanocarrier for Plant-produced Growth Factors Delivery

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[1]Baiyaphytopharm Co., Ltd., Thailand

[2]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330 Thailand

Introduction

Growth Factor, a bioactive molecule discreetly secreted, assumes a pivotal role in cell proliferation. Its applications span various industries, including cosmetics, cell-based meat, and medicine. However, the market price of this indispensable molecule remains prohibitively high due to the intricacies of its production process.



Addressing this economic challenge is plant, an innovative platform engineered to produce biopharmaceuticals with a myriad of advantages. Notable features include sophisticated post-translational modifications, scalability, pathogen and bacterial toxin resistance, and the expeditious production of proteins.

In the cosmeceutical arena, delivering growth factors to subdermal layers poses a formidable challenge due to their low permeability and stability. Nanocarriers emerge as a necessary tool for effective transdermal delivery. This study aims *to develop a nanocarrier platform using natural-based molecules* to encapsulate growth factors derived from *Nicotiana benthamiana.* The primary objectives include assessing encapsulation efficiency and evaluating their delivery capabilities, contributing to the optimization of growth factor utilization in practical applications.

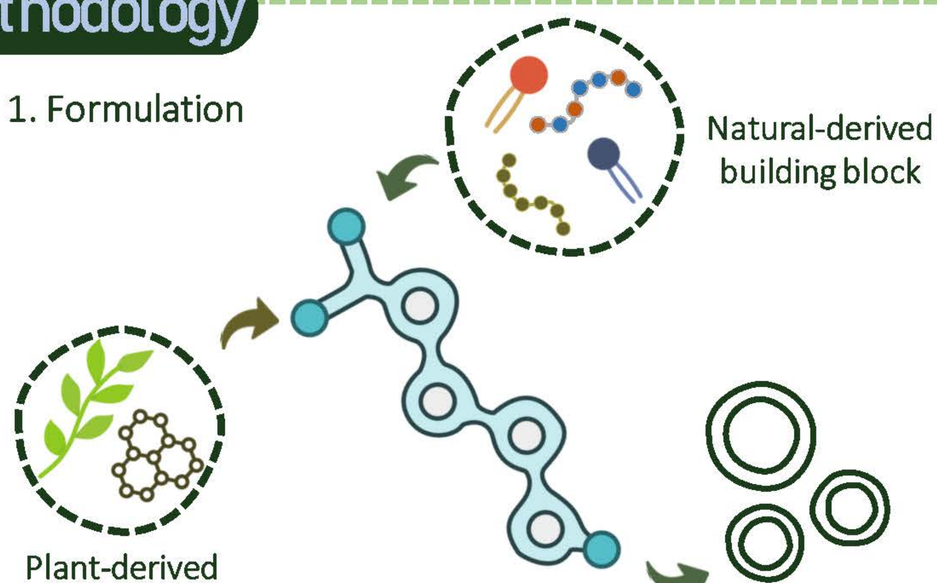
Objectives

To develop nanocarrier as a transdermal delivery system for plant-produced growth factors

Methodology

August - October 2024

1. Formulation



2. Characterization

Size, Zeta potential, Morphology, Encapsulation efficiency, Stability

3. Biological study

Cytotoxicity, cell proliferation study, anti-inflammatory study

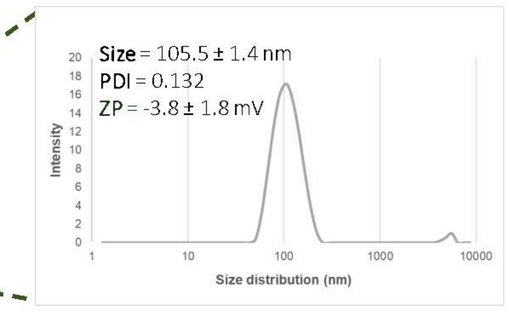
4. Performance evaluation

5. Pre-pilot manufacturing

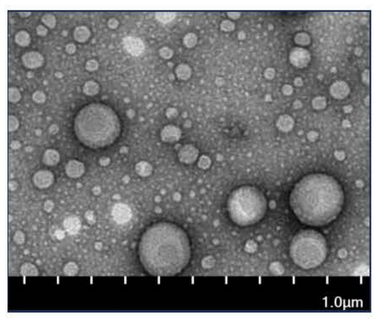
Results and Discussion



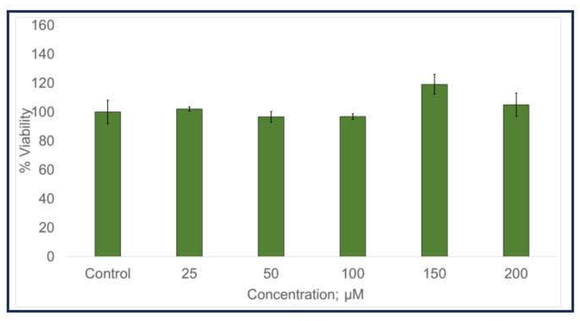
Nano Formulation



Particle characteristic
(Zetasizer Nano ZS)



Particle Morphology
(TEM micrograph)



Cytotoxicity of bare particle
(MTS assay)

Conclusion

Nanoparticle was obtained with a size of 105.5 ± 1.4 nm and a surface charge of -3.8 ± 1.8 mV. From TEM micrograph, the spherical particle was obtained with hollow inside suggesting a vesicle structure. Cytotoxicity test of bare particle shows that they are biocompatible and do not induce cell death up to 200 micromolar.

Acknowledgements

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The production of plant-produced recombinant basic Fibroblast Growth Factor 2 (bFGF2) in tobacco plants

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[2] Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

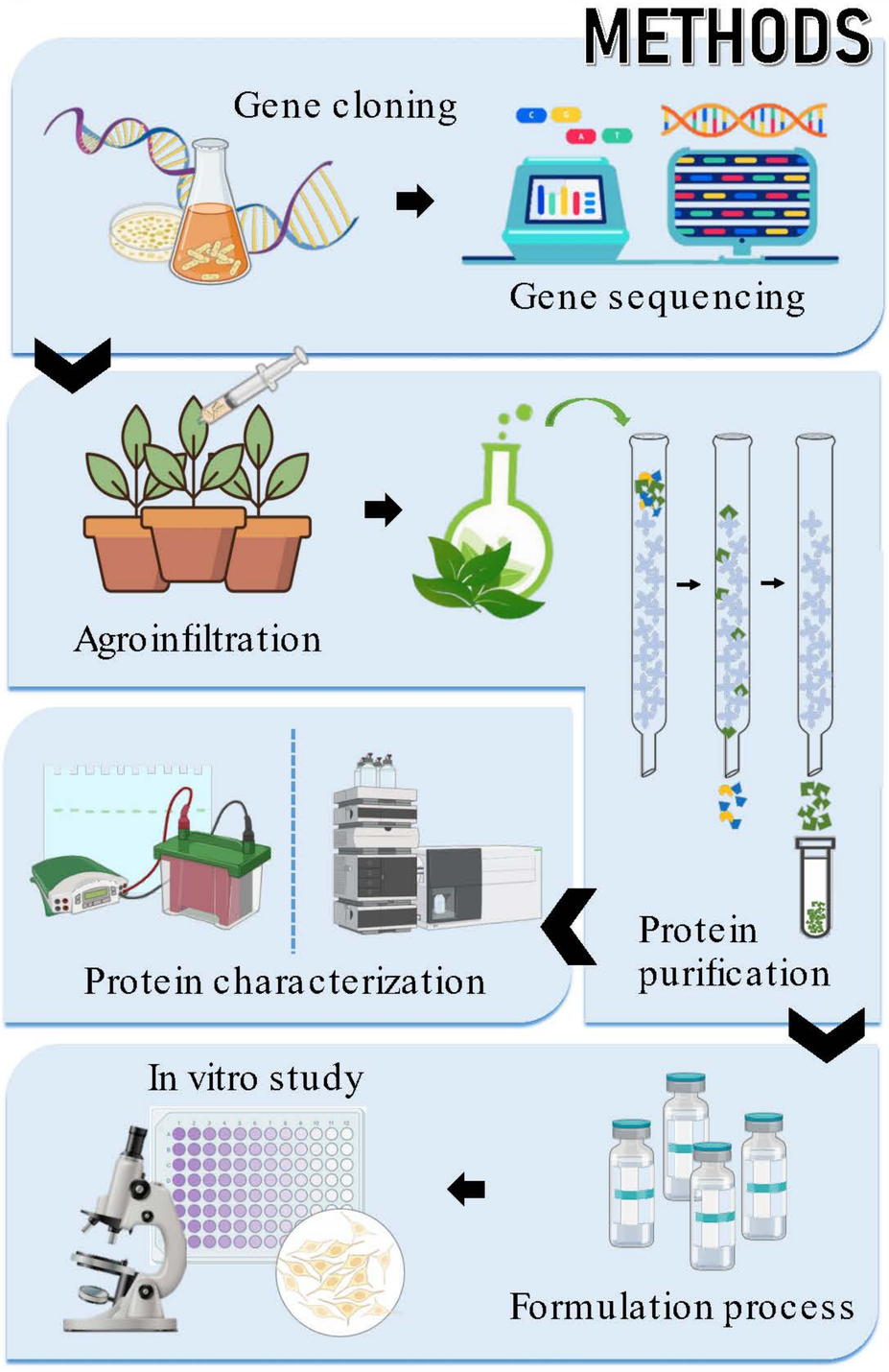
INTRODUCTION

Human basic fibroblast growth factor 2 (bFGF2) is a growth stimulant protein that plays a role in cell proliferation and differentiation process as well as their use in therapy (3). Nowadays, FGF2 is widely applied in various industries. Whether in the medical industry, cosmetics industry and food industry. The production of recombinant FGF2 proteins can be produced in many platforms such as bacteria, yeast or mammalian cell cultures, etc (2). However, the production of FGF2 growth stimulants has a high cost and must be imported from abroad, resulting in products containing growth-stimulating ingredients having high prices. For this reason, plant molecular farming has been developed to replace traditional platform methods. It provides rapid, simple and easy scalability. Additionally, plants can perform the same post-translational modifications as mammalian cells which means they have high biological efficiency of protein. Furthermore, plants are inherently safe because no human pathogens replicate in plants, leading to a notably low pathogen load within the plants and consequently minimizing the risk of process-related contamination (1).

OBJECTIVE

To establish and develop the plant molecular platform process for the high yield and efficacy of bFGF2 production

METHODS



RESULTS & DISCUSSION

To evaluate the expression level of bFGF2 in plants, the gene construct was developed with optimal codon usage and then cloned into geminiviral expression vector pBYR2e (Fig.1) to recombinant protein production via agroinfiltration.

4 days post-infiltration, leaves were harvested. Subsequently, the purification steps were performed. The bFGF2 elute protein was collected and analyzed by western blot technique under reducing condition (Fig. 2)

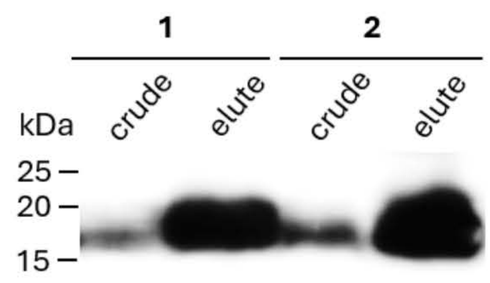


Fig.2 The expression and purification of plant-produced recombinant bFGF2. The protein was observed at 17 kDa as predicted.

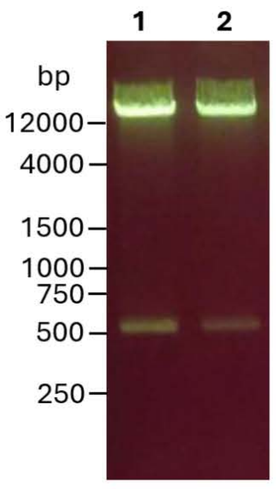


Fig.1 The agarose gel electrophoresis band of the pBYR2e vector containing bFGF2 gene. The bFGF2 insertion gene was cut by specific restriction enzyme site (lower band) and the upper band presented pBYR2e vector.
*1 and 2 represented clone 1 and 2

The results indicated that the plant expression system had a potential to produce the recombinant bFGF2 protein.

CONCLUSION

Successfully modifying the bFGF2 gene was completely cloned into the pBYR2e expression vector, followed by obtaining a high yield expression in tobacco plants, represents a promising step in the development of a plant-based platform for bFGF2 production

ACKNOWLEDGEMENTS

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] and Baiya Phytopharm Co., Ltd.

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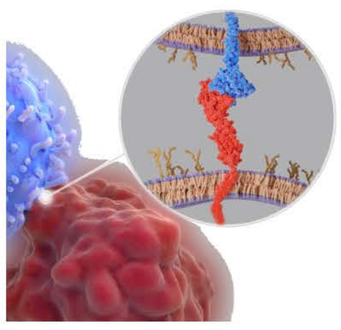
Development of Bispecific Antibodies in Plants for Cancer Immunotherapy

Christine Joy I. Bulaon[1], Panithi Saktrakulkla[1]

[1]Baiya Phytopharm Co., Ltd, Bangkok, Thailand

INTRODUCTION

Immunotherapy has transformed cancer treatment, with immune checkpoint inhibitors to be one example. By receiving antibodies, T cell antitumor response can be restored via checkpoint blockade.



- Interestingly, immune checkpoint inhibitors are now considered standard of care in a wide range of advanced solid tumors.
- Yet, there remains an opportunity to enhance response rates and outcomes from these treatments.

AIMS and HYPOTHESIS

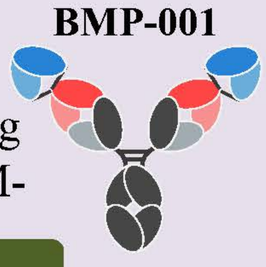
- ✓ **To produce** bispecific monoclonal antibody (bsAb) in *Nicotiana benthamiana*
- ✓ **To test** the efficacy of plant-produced bsAb in mouse tumor model

We hypothesize that the recombinant bsAb can be transiently produced in plants and the plant-derived bsAb elicits superior antitumor activity.

METHODS

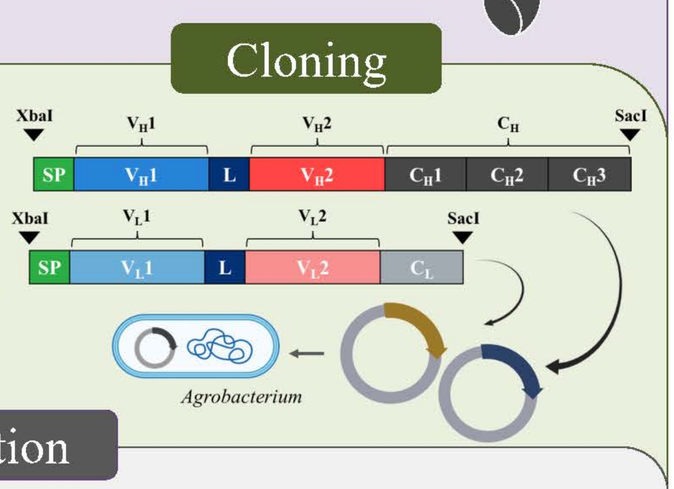
Gene synthesis

- BMP-001 is a full-length bsAb targeting immune checkpoints using plant-produced BPM-003 and BPM-004 blueprints



Cloning

- Overlap PCR for bsAb synthesis
- *Agrobacterium* transformation



Plant infiltration



- Syringe infiltration for small-scale bsAb expression
- Expression in tobacco was evaluated by protein-based assays

RESULTS and DISCUSSION

1. SYNTHESIS and CLONING

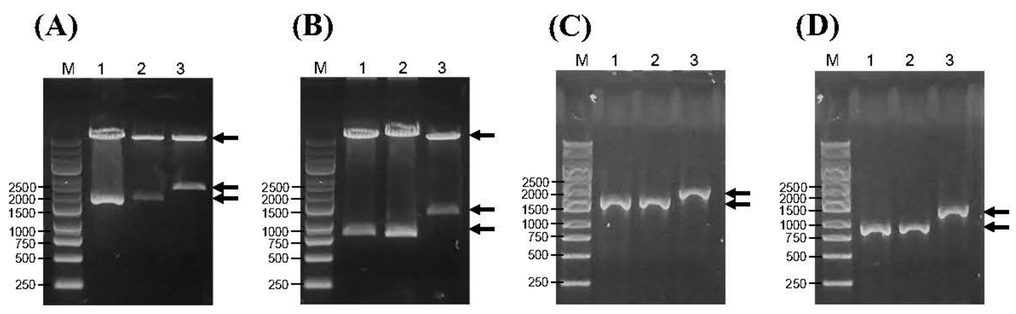


Figure 1. *Restriction enzymes digestion and PCR analysis of expression vectors. M: DNA ladder, 1: BPM-003 HC/LC, 2: BPM-004 HC/LC, 3: BMP-001 HC/LC.*

- The HC (A) and LC (B) gene inserts of BMP-001 and parental IgGs were detected at expected gene sizes and expression vector at about >13,000 bp.
- HC (C) and LC (D) plasmid vectors were detected in *A. tumefaciens* transformants.

2. EXPRESSION

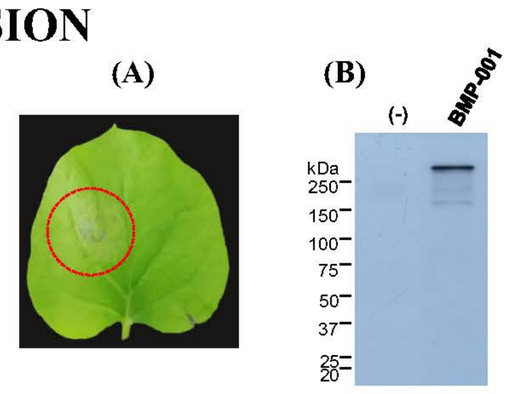


Figure 2. *Transient expression of BMP-001 bsAb in N. benthamiana.*

- Expression of BMP-001 bsAb caused necrosis (A) and the fully-assembled bsAb was detected at expected molecular weight in crude extract (B).

- ✓ The recombinant BMP-001 bsAb was synthesized by overlapping PCR and expressed in plants.
- ! The production and purification of plant-produced BMP-001 will be investigated next.

CONCLUSION

The recombinant bsAb was successfully generated via PCR strategy and produced *in planta* via transient expression.

ACKNOWLEDGEMENTS

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] and Baiya Phytopharm Co., Ltd.

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Plant Produced Anti-IL 6R Antibody and its Role in Inflammatory Diseases

Ashwini Malla[1], Panithi Saktrakulkla[1], Suthira Taychakhoonavudh[1,2]

[1]Baiya Phytopharm Co., Ltd., Bangkok, Thailand

[2]Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand

Introduction

•Interleukin 6 (IL-6), promptly and transiently produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, haematopoiesis, and immune reactions.

•Although its expression is strictly controlled by transcriptional and post transcriptional mechanisms, disregulated continual synthesis of IL-6 plays a pathological effect on chronic inflammation and autoimmunity.

•IL-6 is an inflammatory cytokine which has a critical role in immune processes and is implicated in various diseases, including cancer.

•IL-6 is mainly produced by T cells and macrophages to stimulate the immune response (pro-inflammatory), and an increase in IL-6 levels is an important contributor of the pathogenesis of inflammatory diseases.

•IL-6 plays multiple biological roles in different signaling pathways through the IL-6 receptor (IL-6R) and activates downstream intracellular signaling cascades including the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway.

•Tocilizumab, a mAb binding to the IL-6 receptor (IL-6R), is approved for the treatment of several auto-inflammatory diseases, such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis and is the first approved treatment for chimeric antigen receptor (CAR) T-cell therapy–induced cytokine release syndrome (CRS).

Methods

Gene Identification, Codon Optimization & Synthesis

↓

Cloning into Expression Vector & *E. coli* transformation

↓

Colony PCR, Plasmid Isolation & Confirmation by Restriction Digestion

↓

Transformation of Anti-hIL 6R HC & LC into *Agrobacterium tumefaciens*

↓

Buffer Preparation and Plant Infiltration

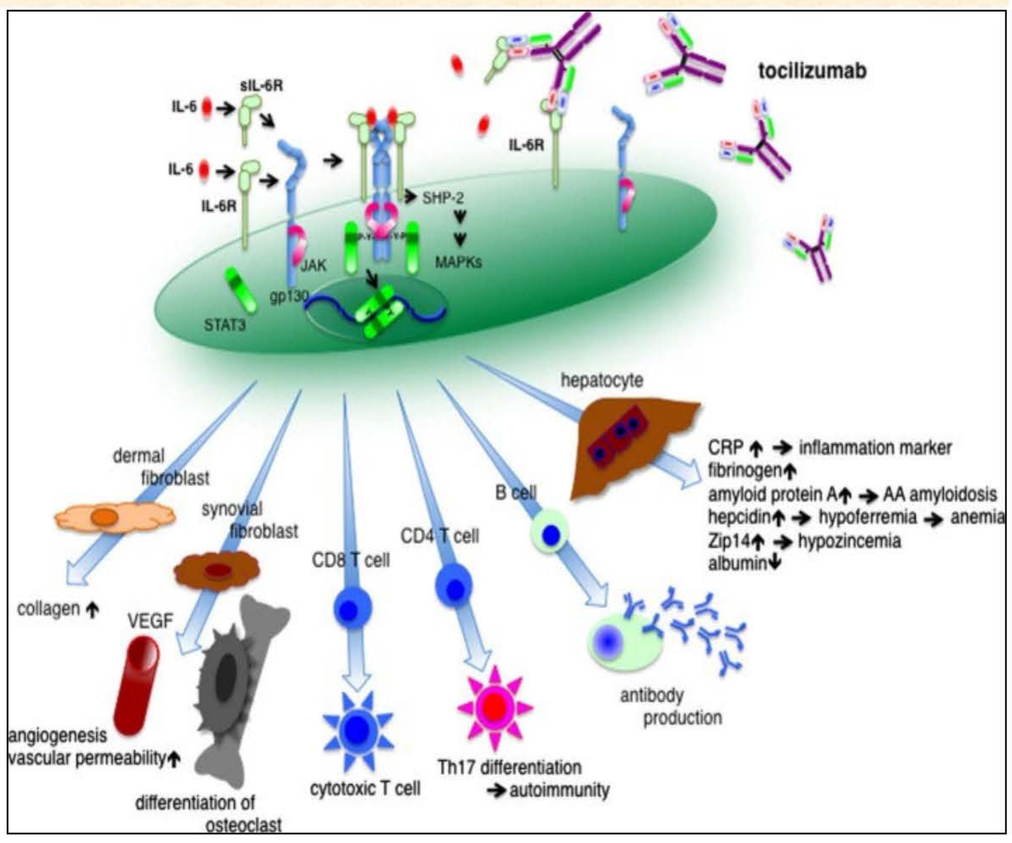
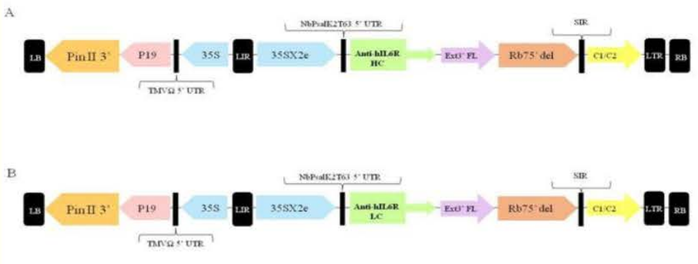


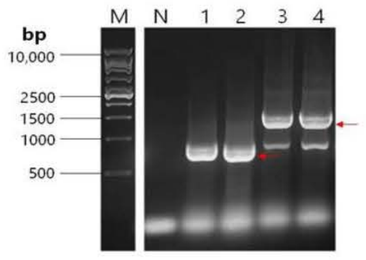
Figure 1: A pleiotropic activity of IL-6

Results

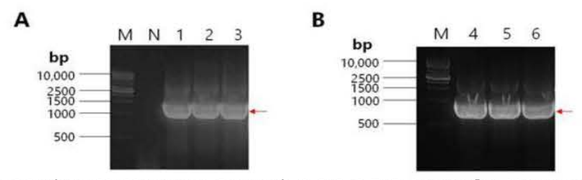
Gene Construct



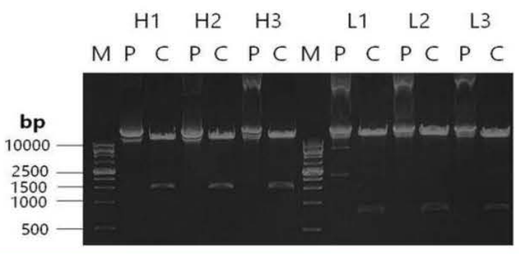
Cloning PCR



Colony PCR



Restriction Digestion



M: Marker; N: Negative Control; 1,2,3,4,5,6: Lanes for Heavy and light chains; H1,H2,H3: Heavy chain; L1,L2,L3: Light chain; P: Plasmid; C: Restriction digested plasmid

Discussion

•The recombinant anti-hIL 6R antibody was successfully cloned by overlapping PCR and transformed into *E. coli* and *A. tumefaciens*.

•*Agrobacterium* clones harboring the HC and LC chains of anti-hIL 6R antibody will be used for *N. benthamiana* plant transformation.

Conclusion

•*Agrobacterium* clones are ready for plant infiltration to analyze the assembling of anti-hIL 6R antibody

Acknowledgement

This research has received funding support from the NSRF *via.*, the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B) [Grant Number B13F660137]

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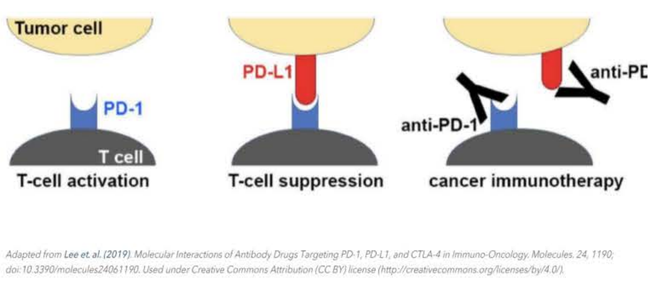
Stability Evaluation of Anti-PD-1 Antibody Pembrolizumab Produced in *Nicotiana benthamiana* Plants

Udomporn Petchthai[1], Panithi Saktrakulkla[1], Suthira Taychakhoonavudh[1,2]

[1]Baiya Phytopharm Co., Ltd., Thailand

[2]Social and Administrative Pharmacy Department, Faculty of Pharmaceutical Science, Chulalongkorn University, Thailand

Introduction



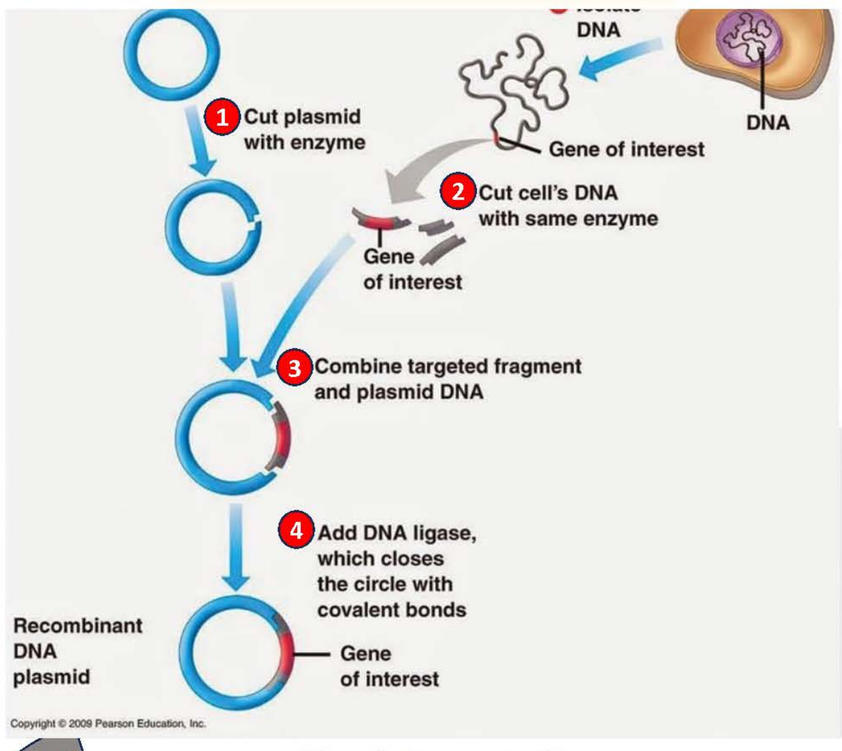
Adapted from Lee et al. (2019). Molecular Interactions of Antibody Drugs Targeting PD-1, PD-L1, and CTLA-4 in Immuno-Oncology. Molecules. 24, 1190; doi:10.3390/molecules24061190. Used under Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

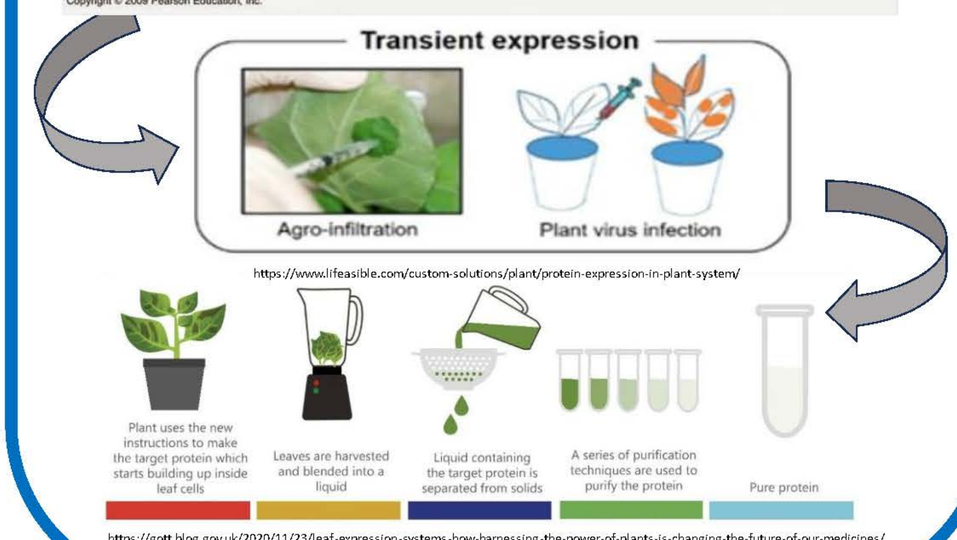
Pembrolizumab is a medication used in the management and treatment of various oncologic conditions. It is in the cancer immunotherapy class of drugs. It works by helping your immune system to slow or stop the growth and spread of cancer cells in the body. Stability testing of drug substances and products allows evaluation of active pharmaceutical ingredient stability or drug product stability.

Objective

The objective of stability testing is to provide evidence on how the quality of a drug substance or drug product varies over a period of time at different temperatures.

Methods

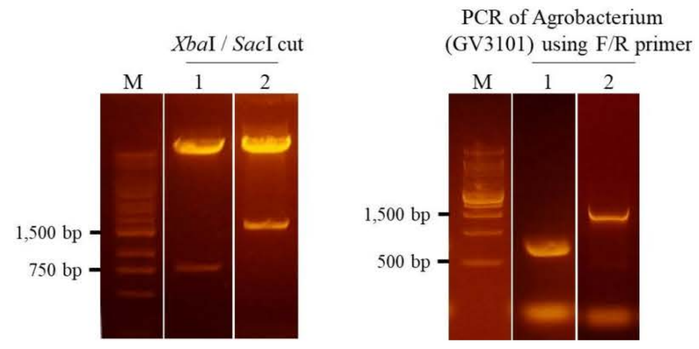




Results & Discussion

❖ Gene design and plasmid construction

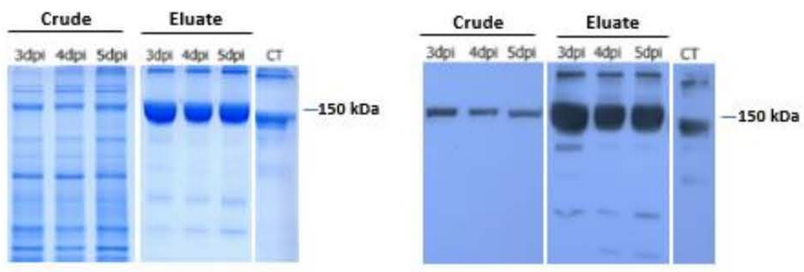
Target gene region was successfully designed and transformed to Agrobacterium.



The presence of target genes were checked by restriction enzyme digestion and colony PCR with specific primers.

❖ Protein expression in plants

Pembrolizumab protein was successfully expression And purified in *N. benthamiana* plants.



The result of Western blot using anti-specific antibody Confirmed that plant-produced Pembrolizumab can be expressed and purified.

Conclusion

- **Anti-PD-1 Antibody Pembrolizumab can be produced in *Nicotiana benthamiana* Plants.**
- **The stability testing and yield maximizing of Anti-PD-1 Antibody Pembrolizumab is designing.**

Acknowledgements

This research has received funding support from;

1. **The NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137]**
2. **Baiya Phytopharm Co., Ltd., Thailand.**

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Effect of Aglycan Version of Plant Produced Pembrolizumab (IgG1) on the Fc Function

Kaewta Rattanapisit and Panithi Saktrakulkla

Baiyaphytopharm Co., Ltd., Bangkok, Thailand

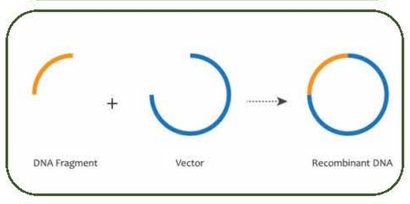
Introduction

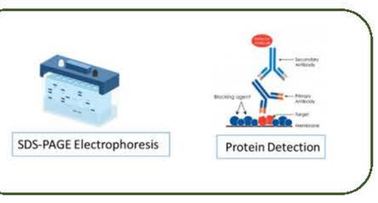
Pembrolizumab is a monoclonal anti-programmed death receptor-1 (PD-1) antibody that has been extensively investigated in numerous malignancies. Pembrolizumab belonging to the IgG4 class of antibody. Glycosylation in the conserved asparagine residue in the heavy chain region of antibodies is one of the most common post translational modifications and an important critical parameter in monoclonal antibody therapeutics. Our preliminary study showed that the aglycosylated IgG1 version of plant produced monoclonal antibody Atezolizumab showed less FcR binding in vitro. Hence, in this proposal, we aim to produce anti-PD-1 IgG4 antibody Pembrolizumab in plants with aglycosylated IgG1 backbone harboring the mutation at N297A in order to assess whether the aglycosylated IgG1 version show less FcR binding activity compared to the original IgG4 version. The aglycosylated IgG1 version of Pembrolizumab will be cloned into plant expression vector and produced in Nicotiana benthamiana plants by transient expression and purified by affinity column chromatography. The purified proteins will be tested for their binding to human FcγRs in vitro. If this aglycosylated IgG1 version reduces the FcγR-binding and effector functions, the IgG1 version of plant-produced Pembrolizumab could make a positive impact on the biotherapeutic development with significantly reduced effector functions.

Objective

- To produce the aglycan version of anti-PD-1 mAb Pembrolizumab in IgG1 format in plants and assess its effect on the Fc receptor binding and effector functions.

Methods

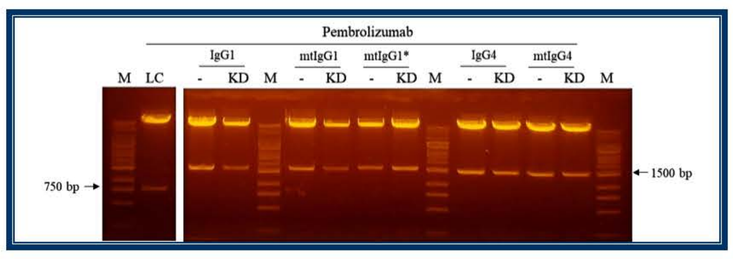
1. Gene Synthesis & Primer design ⇔ LC, HC
2. Plasmid Cloning ⇔ 

3. Agrobacterium Transformation ⇔ 
4. Agro-infiltration ⇔ 
5. Determination of protein expression ⇔ 

Results & Discussion

Cloning of plasmid DNA

The results of plasmid DNA that was cut with XbaI/SacI enzyme. Light and heavy chain showed inserted band at 750bp and 1500bp, respectively.

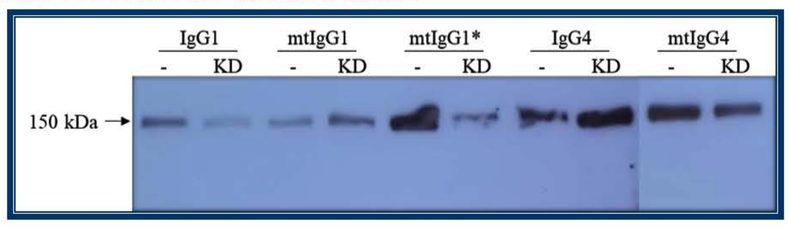


Colony PCR of Agrobacterium (GV3101)

The results of Agobacterium colony PCR using forward/reverse vector primer confirmed correct size of light and heavy chain.

Protein expression in Tobacco leave

The results of western blot using anti IgG Gamma antibody confirmed that co-infiltrated tobacco leave expressed the full combination of antibody at 150kDa, similar with other antibodies.



Conclusions

Several version of glycan in Pembrolizumab can produced in tobacco

Acknowledgements

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] and Baiya Phytopharm Co., Ltd.

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Development of Antibody-cytokine Fusion for Novel Cancer Therapy

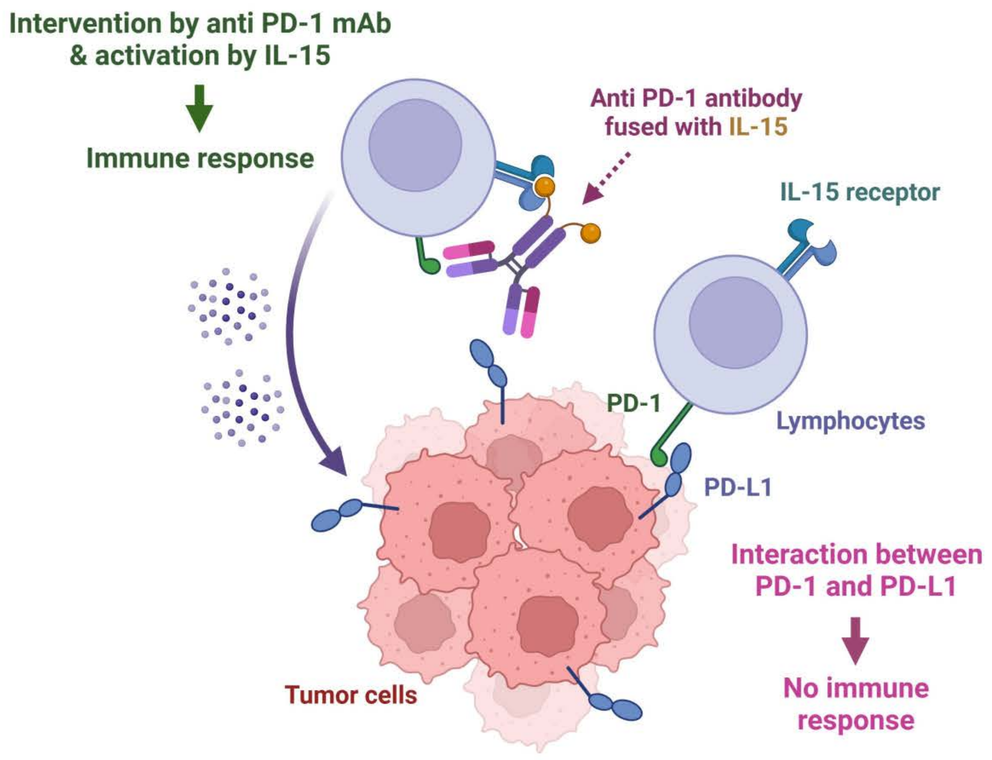
Pipob Suwanchaikasem[1], Kaewta Rattanapisit[1], Christine Joy I. Bulaon[2,3], Panithi Saktrakulkla[1], Jittima Luckanagul[4], Waranyoo Phoolcharoen[2,3]

[1]Baiya Phytopharm Co., Ltd., [2]Center of Excellence in Plant-produced Pharmaceuticals, Chulalongkorn University, [3]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, [4]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University,

สร้างคน ข้ามพรมแดน

Introduction

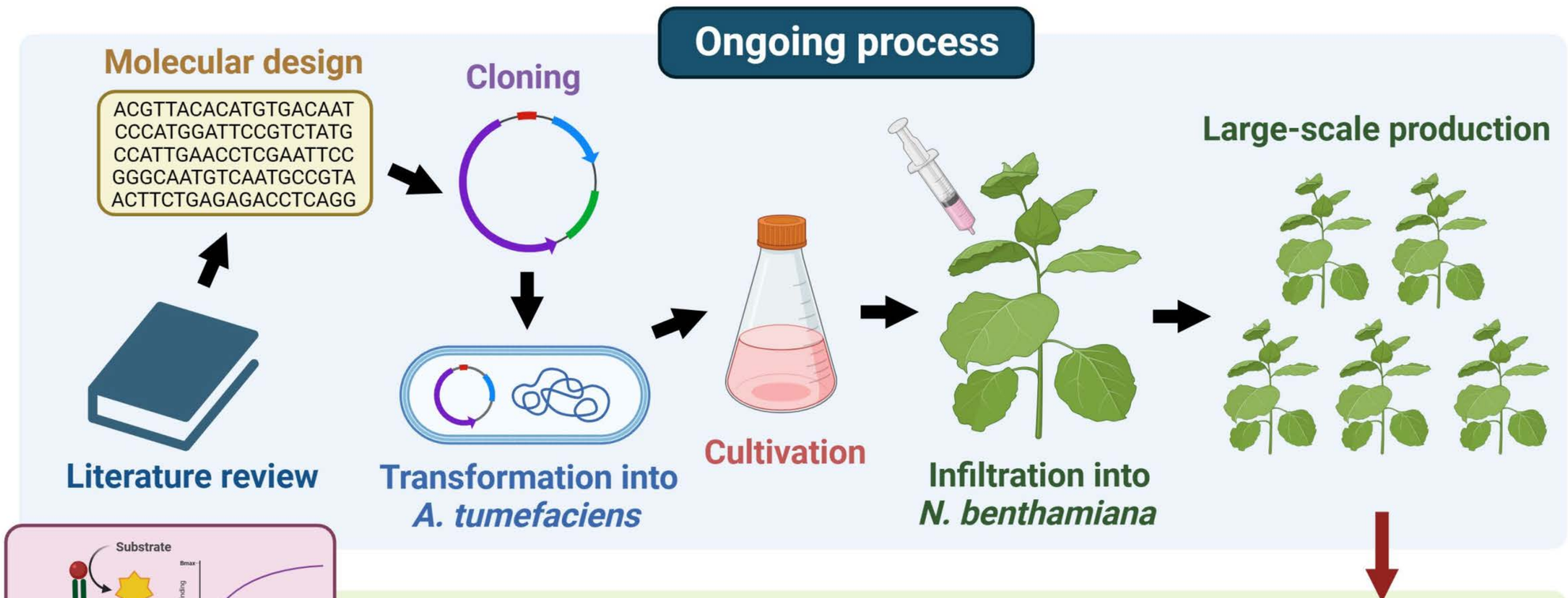
In cancer therapy, monoclonal antibodies (mAbs) that target PD-1 and PD-L1, such as pembrolizumab, nivolumab and atezolizumab, have shown clinical success in treating various cancer types, such as colorectal cancer, small and non-small cell lung cancers, melanoma and esophageal squamous cell carcinoma. However, some patients develop poor responses to those drugs and need combination treatments with radio- or chemotherapy. Immunocytokine (antibody-cytokine fusion protein) is an up-and-coming approach to enhance mAbs efficacy. The mAbs not only block PD-1 and PD-L1 interaction, but also direct cytokines to tumor site, which allow cytokines to mediate biological functions of surrounding immune cells, thereby increasing immune responses against tumor cells.

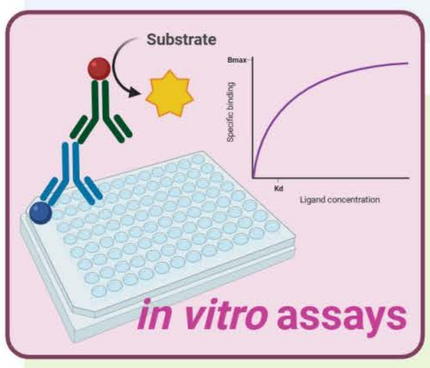


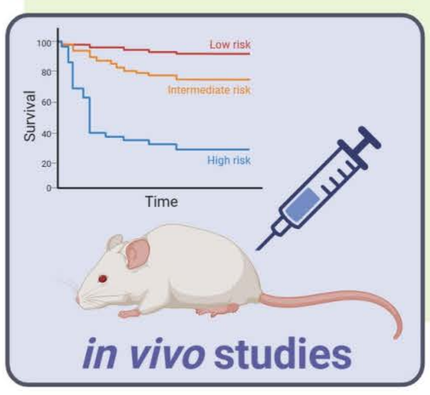
This study aims to develop novel antibody-cytokine fusion by coupling anti-PD-1 mAb with interleukin-15 (IL-15), a potential cytokine to activate cytotoxic T cells and natural killer cells, to synergize immunological functions and reduce adverse effects in cancer treatment.

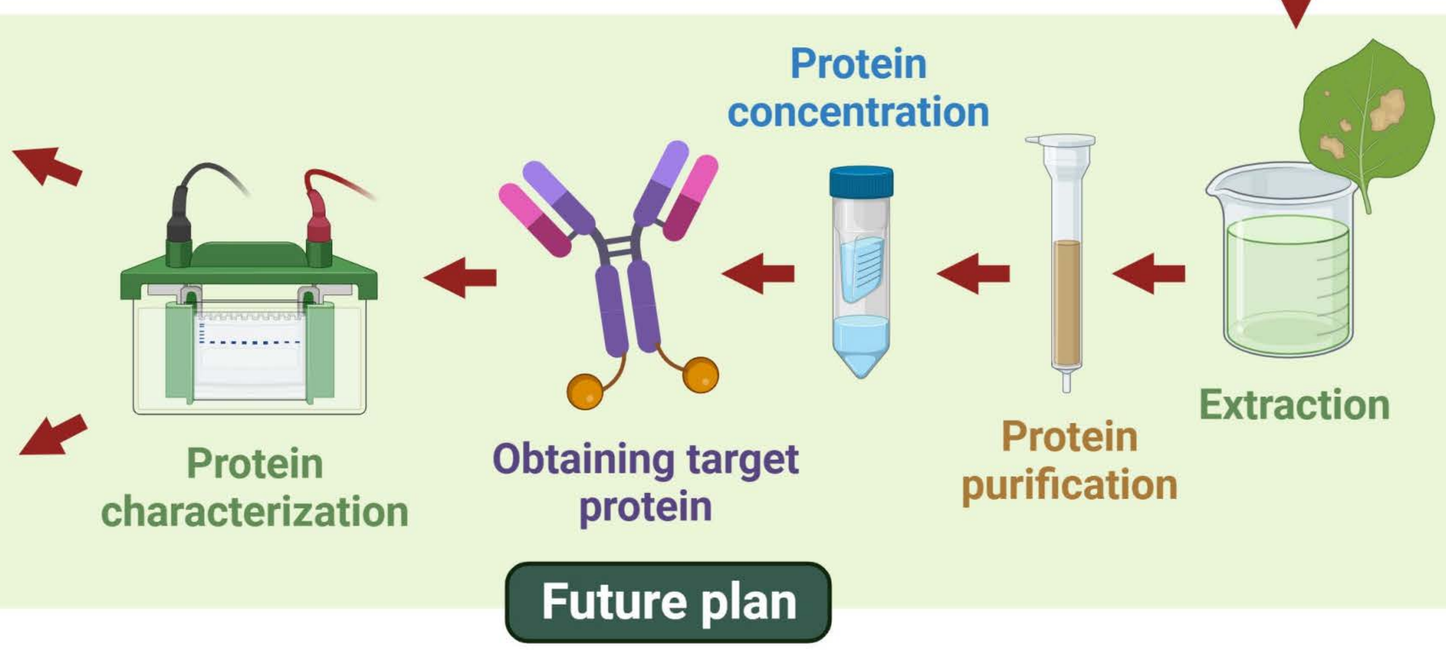
Progress & plan

Ongoing process









Future plan

Acknowledgement: All figures were created with Biorender.com. We thank PMU-B, NSRF (grant no. B13F660137) for financial support.

References:

Kirchhammer N, et al. 2022. *Sci Transl Med*, 14: eabo3605.
Runbeck E, et al. 2021. *Antibodies*, 10: 10.

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Development of pant-produced recombinant protein in cancer and infectious disease treatment

Narach Khorattanakulchai[1], Panithi Saktrakulkla[1], Suthira Taychakhoonavudh[1,2]
[1] Baiya Phytopharm Co., Ltd., Thailand
[2] Faculty of Pharmaceutical Science, Chulalongkorn University, Thailand

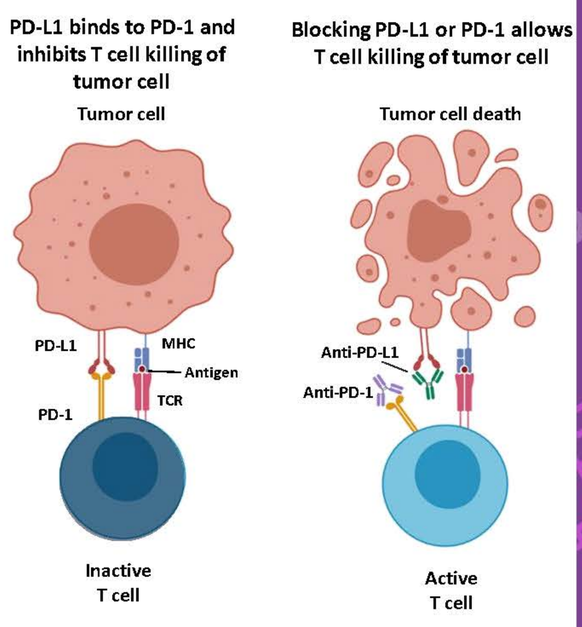
Introduction

Immunotherapy with monoclonal antibodies (mAbs) becomes the new choice to treat cancer. Antagonistic antibodies against immune checkpoint immunoglobulin superfamily molecules, such as CTLA-4, PD-1, and PD-L1, are widely used in clinical benefit and approved by USFDA (Hargadon, K. *et al*., 2018).

However, these antibodies are hardly to access due to the high cost (Tartari, F. *et al*., 2016). Hence, the novel platforms that are cost-effective, scalable, and safe, are required. With low cost for upstream process, easily scale-up, and safe from human pathogen, plant expression system becomes the alternative recombinant protein and antibody productions (Nandi, S. *et al*., 2016). Plant cells efficiently produce complexed proteins, such as antibodies, and perform necessary posttranslational modifications (Fischer, R. *et al*., 2004). Moreover, plant-based technology, especially transient expression, can rapidly produce large amount of protein (D'Aoust, M. A. *et al*. 2010).

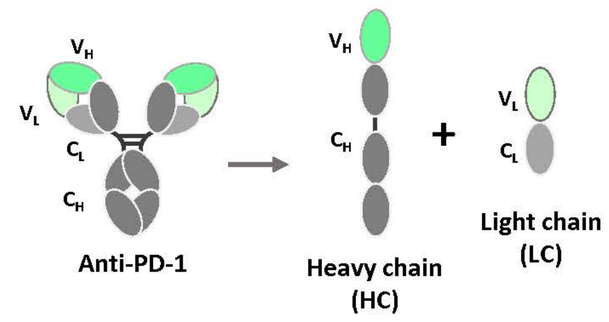
In this study, we developed an anti-PD1 monoclonal antibody (mAb) a promising cancer immunotherapeutic by applying plant-based transient expression in *Nicotiana benthamiana* that enables cost-effective and scalable antibody production.

Mechanism of Anti-PD-1/PD-L1



Methods

1. Design constructs



V_H: Variable region of heavy chain C_H: Constant region of heavy chain
V_L: Variable region of light chain C_L: Constant region of light chain

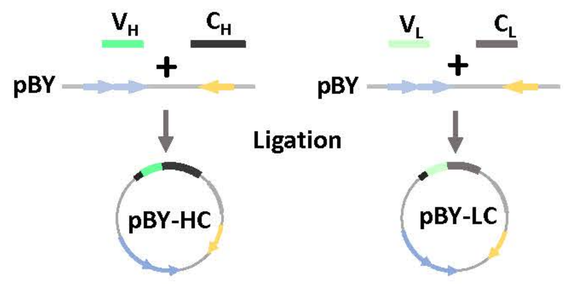
2. Cloning

2.1 Codon Optimization & Gene synthesis



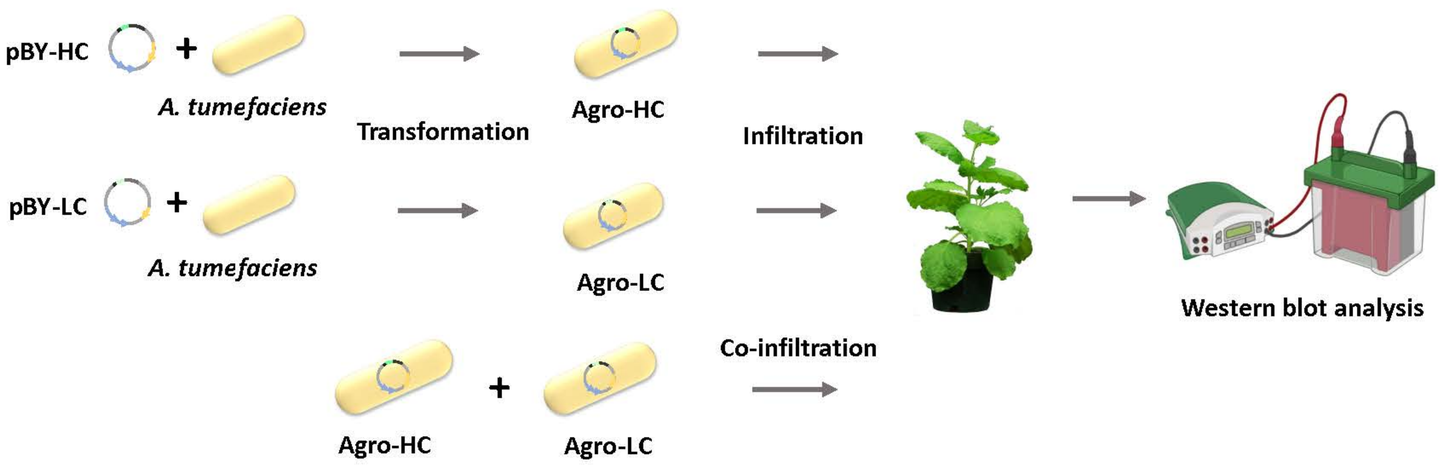
The codon optimized of V_H and V_L genes were synthesized in pUC vectors.

2.2 Genes and expression vector ligation

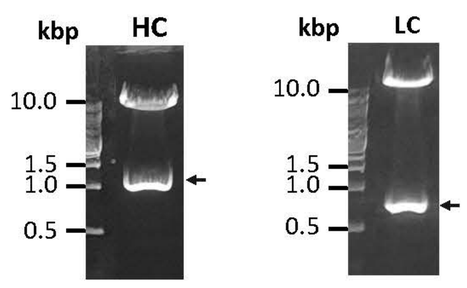


The digested V_H, V_L, C_H, C_L genes, and expression vector (pBY) were ligated.

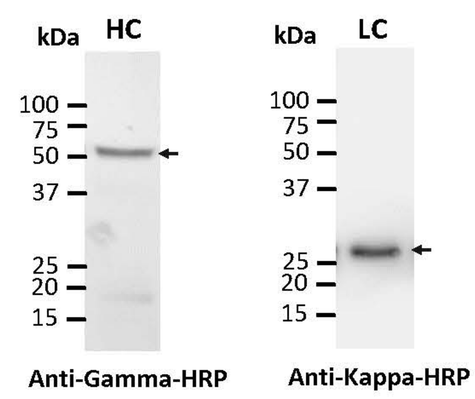
2.3 Agrobacterium transformation and preliminary expression



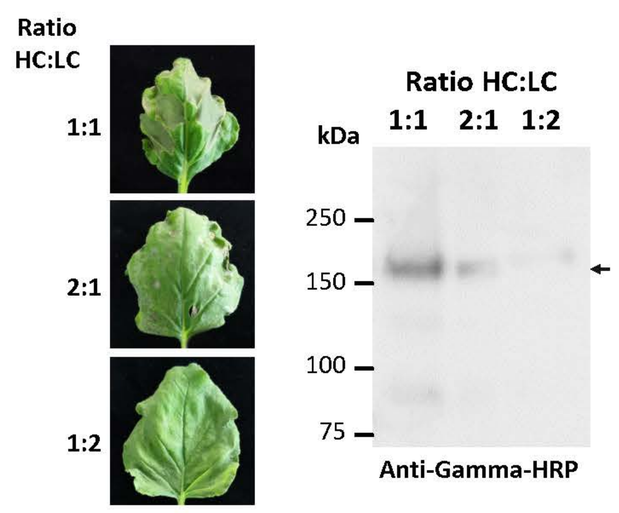
Results



Both HC and LC constructs were successfully ligated in to pBY vectors.



The preliminary expression of both HC and LC were successfully expressed in *N. benthamiana.*



The co-expression of both HC and LC were successfully expressed full antibody in *N. benthamiana.*

Conclusion

Anti-PD-1 is successfully expressed in N. benthamiana and the optimum ratio of Agro-HC:Agro-LC for co-infiltration is 1:1.
The optimization for high yield will be further investigate.

Acknowledgements

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137]

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Expression and in vitro study of plant-produced human interleukin-15

Chalatorn Charnasatabut[1] and Waranyoo Phoolcharoen[1,2]

[1]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceuticals Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[2]Center of excellence in Plant-produced Pharmaceuticals, Chulalongkorn University, Bangkok 10330, Thailand

Rationale

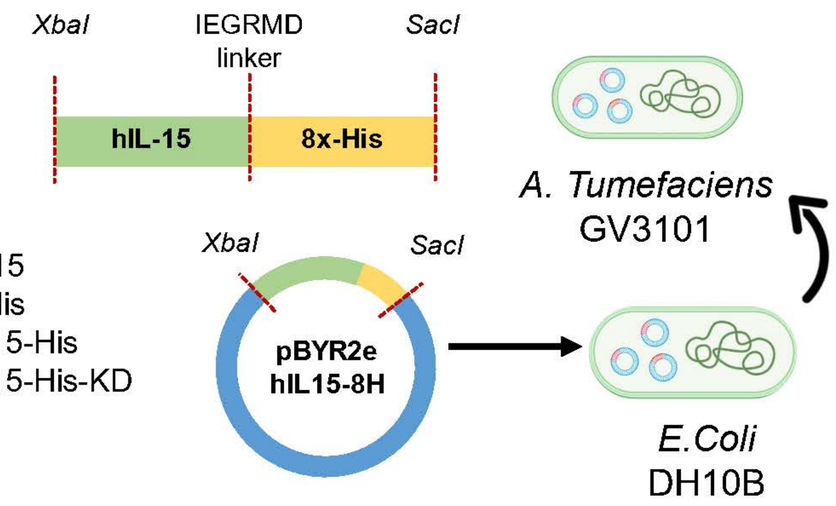
- Immunotherapy is a type of cancer treatment that uses patients' immune systems to interact with cancer by improving the immune system to find and destroy cancer cells. At first, stimulatory cytokines such as interferon alpha (IFN-α) and Interleukin-2 (IL-2) were focused on immune response enhancement in clinical trials. Result in IL-2 approval by FDA for metastatic melanoma and metastatic renal carcinoma treatment. Unfortunately, IL-2 is related to several serious side effects involving capillary leak syndrome, hypotension, hypoxia, and oliguric renal failure. These concerns led to the development of new IL-2-like immunotherapy but fewer adverse effects.

- Interleukin-15 (IL-15) belongs to the same family as IL-2. This pleiotropic cytokine has a major role in the promotion of B cells to produce immunoglobulin, activation and survival of CD8+ T cells, γδT cells, natural killer (NK) T cells, and NK cells which improve the anti-tumor activity of immune cells. The formation of IL-15/IL-15R complex lead to similar signal transduction by IL-2 and IL-15 that relates to common Janus kinase (JAK) and signal transducer and activator of transcription (STAT) signaling molecules. Several studies in murine and monkey models indicated that IL-15 could be a potential therapeutic protein to treat cancer.

- There are many expression platforms that can be used for therapeutic recombinant protein production. Some factors can be considered for choosing the most suitable platform; yield, post-translational modification (PTM), growth rate, cost, and safety. For this study, the plant expression platform is selected due to the fast growth rate, ability of PTM such as glycosylation, low-cost operation, easy to optimize the growth condition and scale-up the production. To express the protein transiently, *Agrobacterium* will be involved as a carrier to transfer the expression vector that contains the IL-15 gene into the plant using the vacuum agroinfiltration. After that, the infiltrated plant will be able to produce the protein. *Nicotiana benthamiana* is the Australian tobacco plant that will be used in this study to produce the recombinant hIL-15. According to the properties of this species, tobacco can give a high biomass yield, capability of rapid scale-up because of high seed production. That makes *N. benthamiana* appropriate for recombinant production

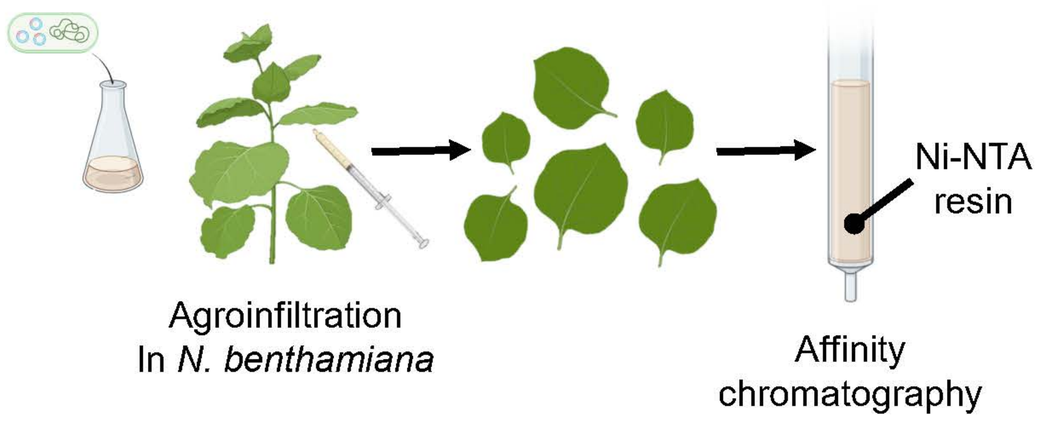
Methodology

Expression vector construction



- His-hIL-15
- hIL-15-His
- SP-hIL-15-His
- SP-hIL-15-His-KD

Protein production & purification



Protein analysis

- SDS-PAGE
- Western blot
- Bradford's assay



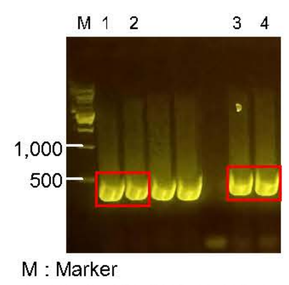
Biological activity

- PBMCs proliferation
- K_D determination



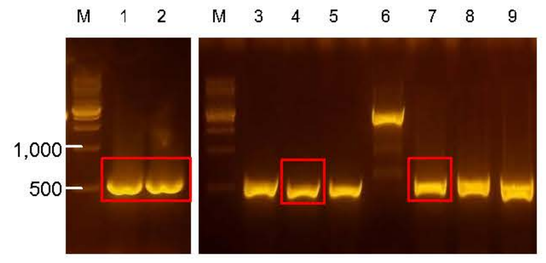
Result & Discussion

(A) Colony PCR of *E. coli*



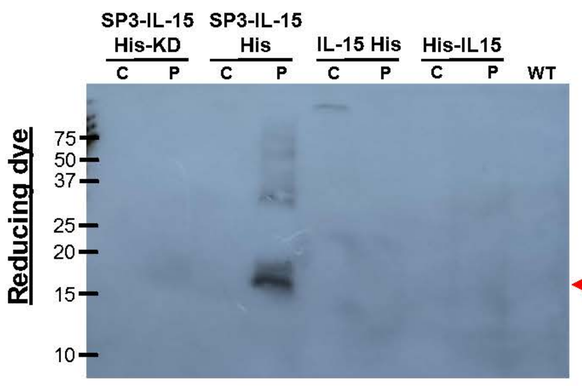
M : Marker
1 : His-IL-15/pBY2eK-1(Colony PCR)
2 : His-IL-15/pBY2eK-2(Colony PCR)
3 : SP-IL-15-His /pBY2eK-1(Colony PCR)
4 : SP-IL-15-His /pBY2eK-2(Colony PCR)

(B) Colony PCR of *A. tumefaciens*

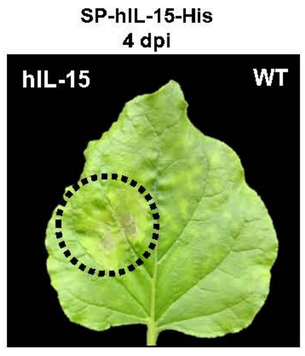


M : Marker
1 : His-IL-15/pBY2eK-1(Colony PCR)
2 : His-IL-15/pBY2eK-2(Colony PCR)
4 : SP-IL-15-His /pBY2eK-1(Colony PCR)
7 : SP-IL-15-His /pBY2eK-4(Colony PCR)

(C) Protein expression of hIL-15



(D) Leaf after infiltration



- As the result of colony PCR, the band estimated to be around 500 bp which is the size of hIL-15 fused with Histidine-tag
- For the protein expression analysis by western blot, the expression of SP3-IL15-His can be seen, the size is around 17 kDa
- The phenotype of the leaf is affected by the expression of the protein > **Necrosis**

Conclusion

To produce the recombinant protein IL-15 using *N. benthamiana* as an expression platform, the first step is to construct the expression vector which is successful. The vector was transformed into agrobacterium and was injected into the leaf. The proteins were extracted and analyzed by western blot. SP3-hIL-15-His showed the highest expression compared to other constructs.

The next step is to upscale the hIL-15 production, extract, and purify the protein. Then, plant-produced hIL-15 will be analyzed and quantified before testing its biological activity.

Acknowledgement

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137]

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The effect of plant-produced recombinant Insulin Like Growth Factor-1 (IGF-1) in differentiation of mammalian cell lines.

Kittinop Kittirotruji,[1,2] Waranyoo Phoolcharoen[1,2]

1 Baiya Phytopharm Co., Ltd., Bangkok, Thailand

2 Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

Introduction

Insulin-Like Growth Factor, the polypeptides produced in animals' cells, plays a major role in cell proliferation and reparation. There are two subtypes of the Insulin-Like Growth Factor comprising IGF-1 and IGF-2. IGF-1 is consisting of 70 amino acids with A and B chains connected with disulphide bonds which is a mediator of growth hormone's anabolic and mitogenic activity (1). It is also used as an effective treatment in several diseases such as diabetes type 1 and 2, wound healing, and short stature syndrome (2). In recent years, the growth factor has become an essential substance in many industries especially in the cultivated meat market. They are using the growth factor to cultivate mammalian cells of interest in a bioreactor that eventually form into the edible meats. Although the needs of the growth factors in this market have been rising, the price of the growth factor in the market is still unaffordable due to the expensive expression process, therefore, this research will produce the IGF-1 in plant expression system using a cheaper bioreactor and test the efficacy in mammalian cell lines.

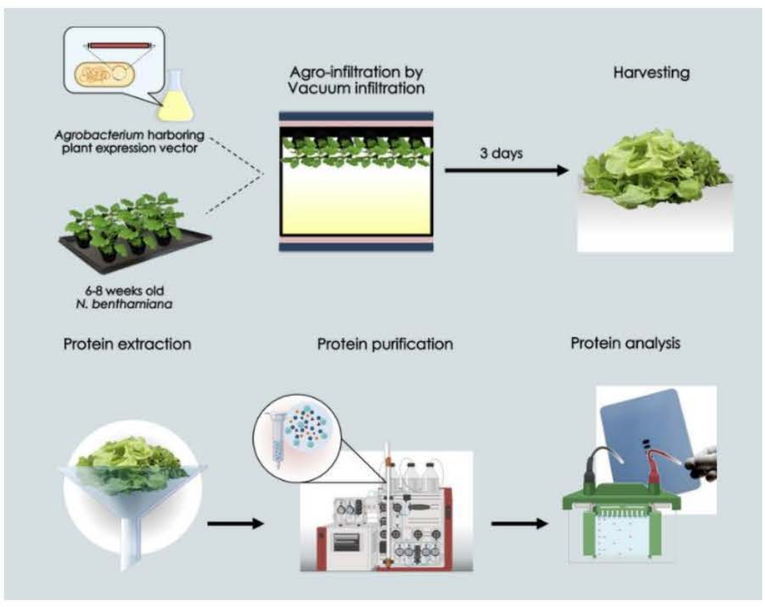
Rational/Promblem statement

The results of this research will ensure the feasibility of using plant expression system to produce recombinant IGF-1 as a cost-effective method. Moreover, the production process could be developed to scale-up and become commercialized in order to serve the market demand in the future.

Methods

Expression of recombinant human IGF-1 in plant

1. Sequence selection
2. Gene cloning
3. Agrobacterium infiltration into N.benthamiana
4. Purification and formulation



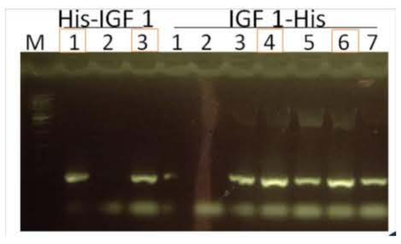
Conclusion

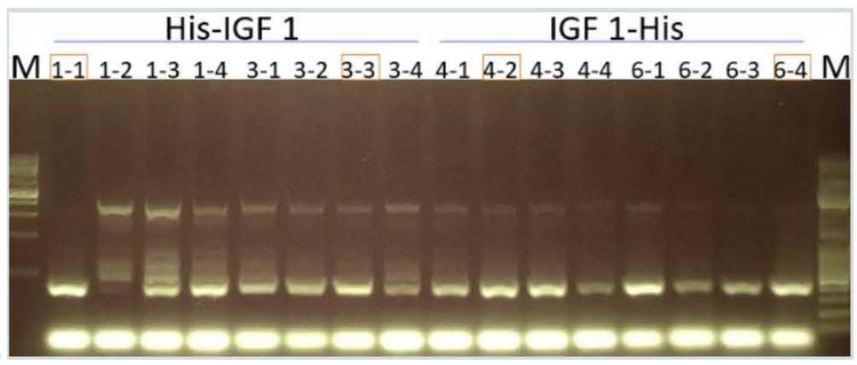
The proof of concept in plant-produced IGF-1 was approved by the results, then we plan to further test the proliferation efficacy of plant-produced IGF-1 by using cell lines.

Results & Discussion

We conducted the E.coli transformation in order to select the proper sequence to infiltrate in *N.Benthamiana* for plant-produced IGF-1. then we use the selected agrobacterium to conduct day optimization of proteins production in *N.benthamina* to see whaether what day is the best yield of IFGF-1 production in plant.

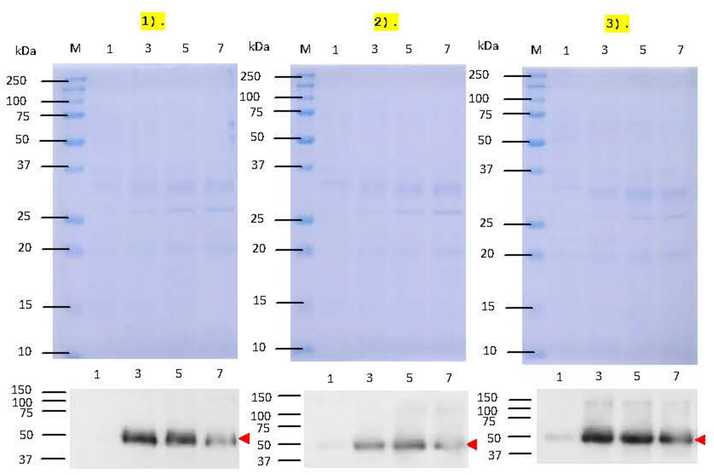
The band of each E. coli clones containing pBY2Re vector that was verified by colony PCR.

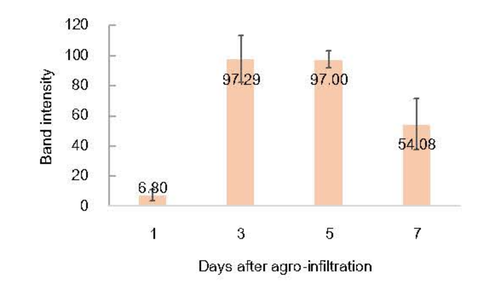


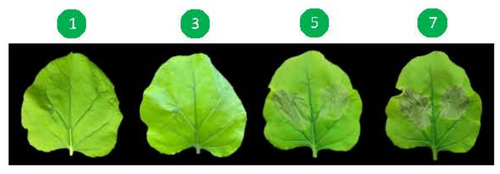


The band of each A. tumefaciens clones containing pBY2Re vector that was verified by colony PCR as detailed. The label in red represented the correct clone was selected to do the further step.

Day Optimization







References

1. Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Pathol. 2001 Oct;54(5):311-6. doi: 10.1136/mp.54.5.311. PMID: 11577173; PMCID: PMC1187088.
2. Iranpoor H, Omidinia E, Vatankhah V, Gharanjik V, Shahbazi M. Expression of Recombinant Human Insulin-like Growth Factor Type 1 (rhIGF-1) in Escherichia coli. Avicenna J Med Biotechnol. 2015 Jul-Sep;7(3):101-5. PMID: 26306149; PMCID: PMC4508332.

Acknowledgements

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Production and Characterization of Monoclonal Antibodies Against BotulinumToxin Produced in *Nicotiana benthamiana*

Kornchanok Sangprasat [1,2] , Panithi Saktrakulkla [2], Waranyoo Phoolcharoen [1,2]

[1]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

[2]Baiya Phytopharm Co., Ltd., Bangkok 10330, Thailand

INTRODUCTION

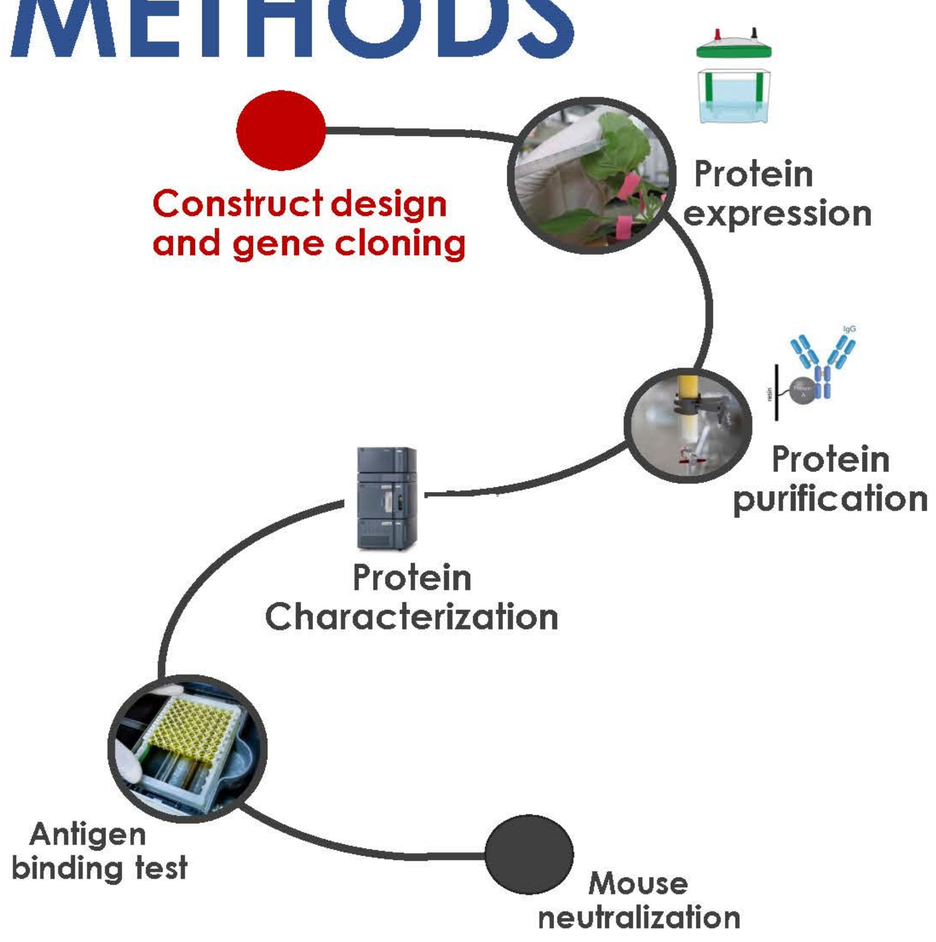
Botulism is a neuroparalytic syndrome resulting from the systemic effects of **botulinum neurotoxin** (BoNT). It is the most potent protein toxin ever characterized. Symptoms of botulinum toxin exposure can range from headache, blurred vision, respiratory paralysis, and death. Currently, it still has serum therapy from animal sources, but it has low specific activity, batch-to-batch variability, disease risk due to pathogen transmission, and the potential for life-threatening hypersensitive immune responses to administered non-self antibodies. These problems can be solved by using genetic engineering techniques such as a monoclonal antibody (mAb).

Plants are considered an interesting alternative for therapeutic protein production because it includes low production costs, scalability, no risk of contamination by human pathogens, and the ability to produce a complex protein with post-translational modifications.

OBJECTIVE

- To produce anti-BoNT antibodies by using *Nicotiana benthamiana*.

METHODS



RESULT AND DISCUSSION

The amino acid sequences of anti-BoNT mAbs were retrieved and codon optimized. The heavy chain (HC) and light chain (LC) genes were commercially synthesized and fused to the constant domains of human IgG1 gamma chain and kappa chain, respectively. The full-length anti-BoNT HC and LC were cloned into pBY2eK geminiviral vector were then transferred into *Escherichia coli* shows the size of the interest gene of HC at 1,500 bps and LC at 750 bps (Fig. **1**) and confirmed by using restriction enzyme (XbaI/SacI) (Fig. **2**) before transformed into *Agrobacterium*. (Fig. **3**) The screening of transformed colonies was carried out by colony PCR with specific primer pairs

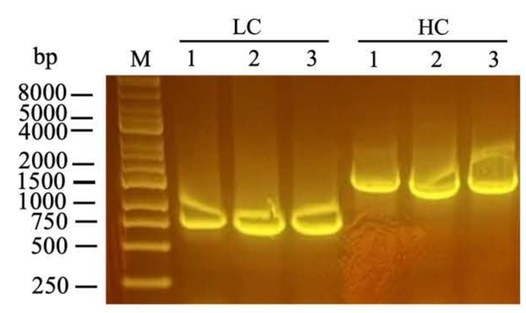


Figure 1. Colony PCR of transformed *E. coli* containing anti-BoNT genes

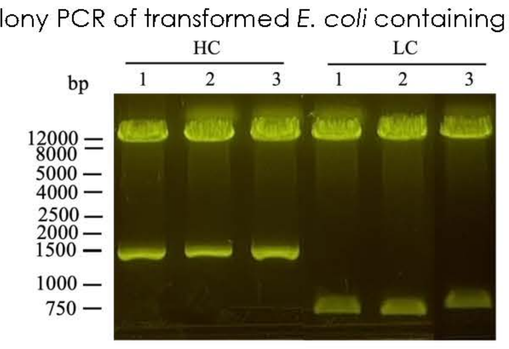


Figure 2. The band of anti-BoNT genes fragment that was cut with restriction enzyme

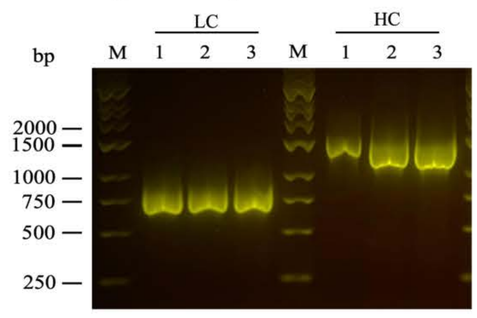


Figure 3. Colony PCR of transformed *Agrobacterium* containing anti-BoNT genes

CONCLUSION

In this experiment, 3 types of monoclonal antibodies against botulinum toxins A and B were produced using the transient expression technique in *N. benthamiana*. There were cloned into the geminiviral vector before transferred into *Agrobacterium*. Furthermore protein expression, purification and characterization will be explored in the future.

ACKNOWLEGEMENT

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137], the scholarship from Chulalongkorn University, The Second Century Fund (C2F), year 2022 and Baiya Phytopharm Co., Ltd.

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Development of *Nicotiana benthamiana* as a Plant-produced Anti-cobravenom

Sarocha Vitayathikornnasak[1,2], Wanatchaporn Arunmanee[2], Panithi Saktrakulkla[1], Kanokporn Pothisamutyothin[2], Waranyoo Phoolcharoen[1,2]
[1] Baiya Phytopharm Co., Ltd., Thailand
[2] Faculty of Pharmaceutical Science, Chulalongkorn University, Thailand

Objective

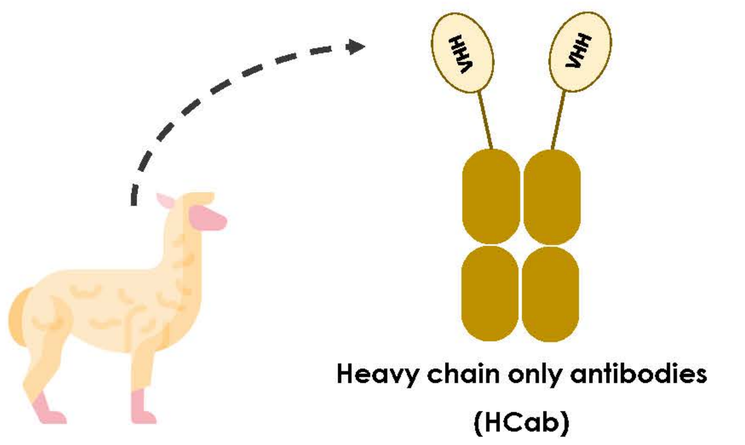
This research aimed to study the expression of produced V_HH-FC into two types which specific to a-Neurotoxin (V_HH-NTX-FC) and Phospholipase A2 (V_HH-PLA_2-FC) in *Nicotiana benthamiana*. The plant-based platform was optimized for the day post infiltration. After attaining the antibody by purifying with protein A chromatography, the test for antigen binding was further analyzed.

Introduction



Snakebite envenomation is a significant health problem in Africa, Asia, and Latin America, especially in South-East Asia, contributing to nearly 70% of global snakebite deaths. Enhancing the quality, efficiency, safety, and affordability of antivenoms is crucial.

The Cobra snake, belonging to the Naja genus in the Elapidae family, produces venom containing a-Neurotoxin (NTX) from the Three-finger toxin (3FTX) group, causing neurotoxic effects. Phospholipases A2 (PLA2) also play a major role, leading to paralysis, respiratory failure, cardiac arrest, and death in Cobra envenomation.



HCab **naturally** produced in shark and camelid species.

The antigen-binding portion of heavy-chain-only antibodies (HCab) is the single variable domain of the heavy chain (V_HH) fragment. Chimeric nanobody-heavy chain antibodies, combining V_HH with the FC region of Human IgG, enhance half-life and are promising for biomedical research, diagnosis, and therapy.

Transient plant-based platforms for antibody production offer advantages like cost-effectiveness, rapid production, and sustainability. Plant-produced monoclonal antibodies (mAbs) show efficacy in medical applications such as HIV, breast cancer, and Covid-19.

Method



Result

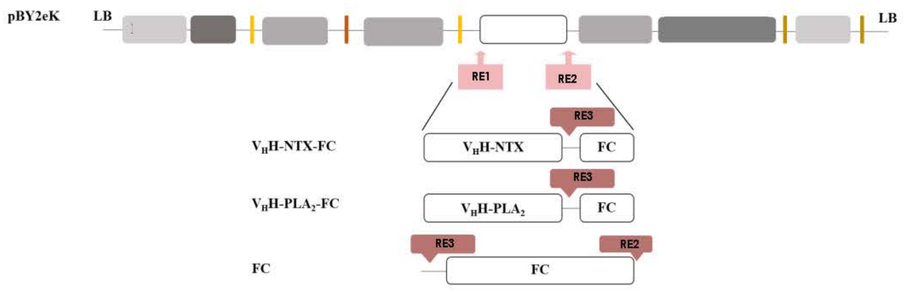
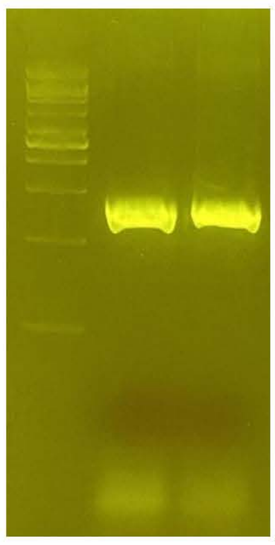


Figure 1. Construct of V_HH-NTX-FC and V_HH-PLA_2-FC genes in geminiviral vectors.



S1: V_HH-NTX-FC
S2: V_HH-PLA_2-FC

Both sequence of V_HH-NTX-FC and V_HH-PLA_2-FC were optimized and completely cloning and transform to E. Coli. the variable region was ligated with constant region of human IgG1 heavy chain CH2 and CH3 (Fc) and linked into geminiviral vector pBY2eK-HC resulting in pBY2eK-VHH-FC.

Acknowledgements

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The Impact of different N-glycan characterizations of plant-produced SARS-CoV-2 RBD with Fc fusion protein on the immune response

Theerakarn Srisangsung[1,2], Panithi Saktrakulkla[2], Waranyoo Phoolcharoen[1,2]

[1]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[2]Baiya Phytopharm Co., Ltd., Bangkok 10330, Thailand

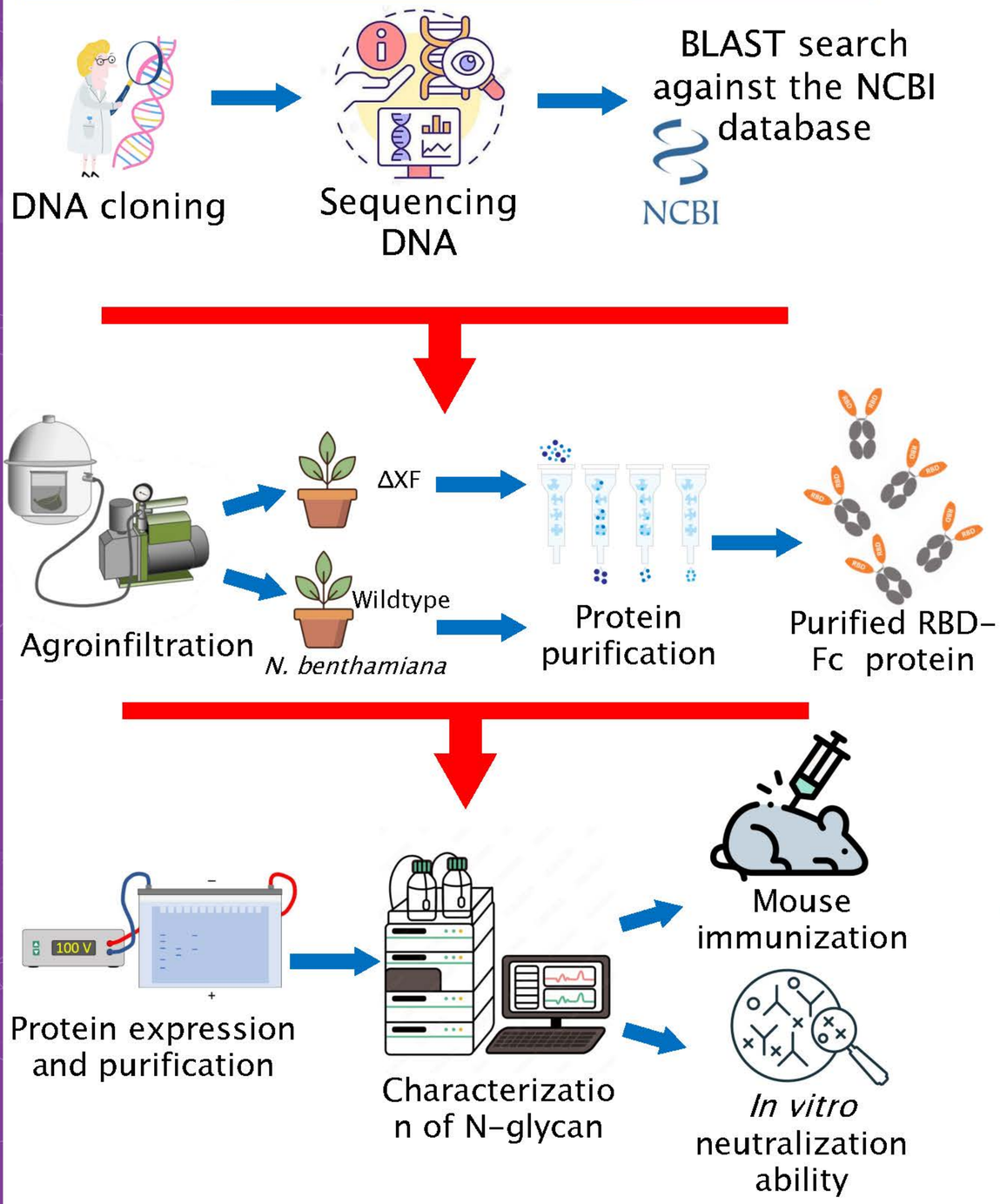
INTRODUCTION

- RBD-Fc is a fusion protein composed of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and the Fc fragment of human IgG1
- RBD-Fc is a fusion protein composed of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and the Fc fragment of human IgG1. It has been used in the development of subunit vaccines against COVID-19.
- *Nicotiana benthamania* has been used as beneficial plant platforms to manufacture antibodies in leaves.
- N-Glycosylation is a type of post-translational protein modification that includes the attachment of carbohydrates (glycans) to proteins and lipids.
- The majority N-glycosylation sequence is Asn-X-Thr/Ser, where X can be any amino acid except Pro.
- This alteration takes place in the endoplasmic reticulum (ER) and is then changed further in the Golgi complex.
- Plant and mammalian glycosylation are different which GnGnXF structures are the most common in plant-produced protein.
- *N. benthamiana* ΔXF plants. are N-glycosylation engineered plant which are knocked down of β1,2-xylosyl- and core α1,3- fucosyltransferases.

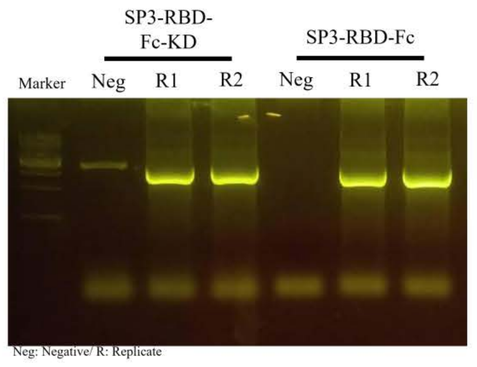
OBJECTIVE

To evaluate the effect of plant-produced SARS-CoV-2 RBD with Fc fusion proteins which were modified from different N-glycosylation to immune response

METHODS

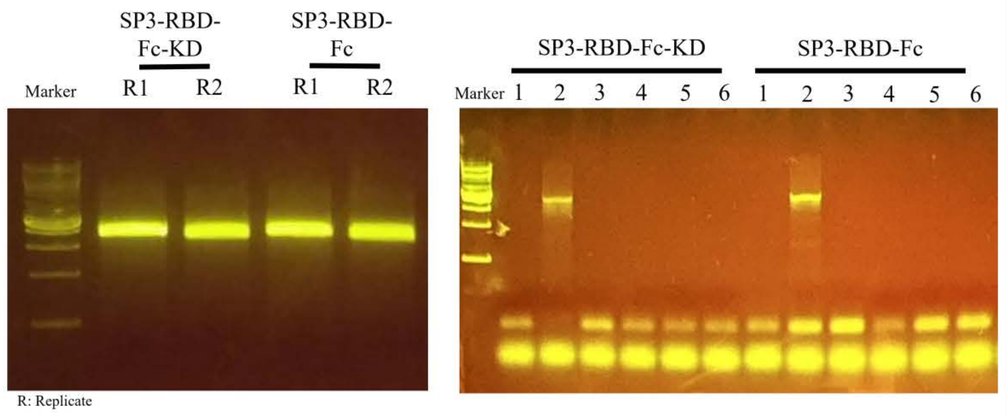


RESULT AND DISCUSSION



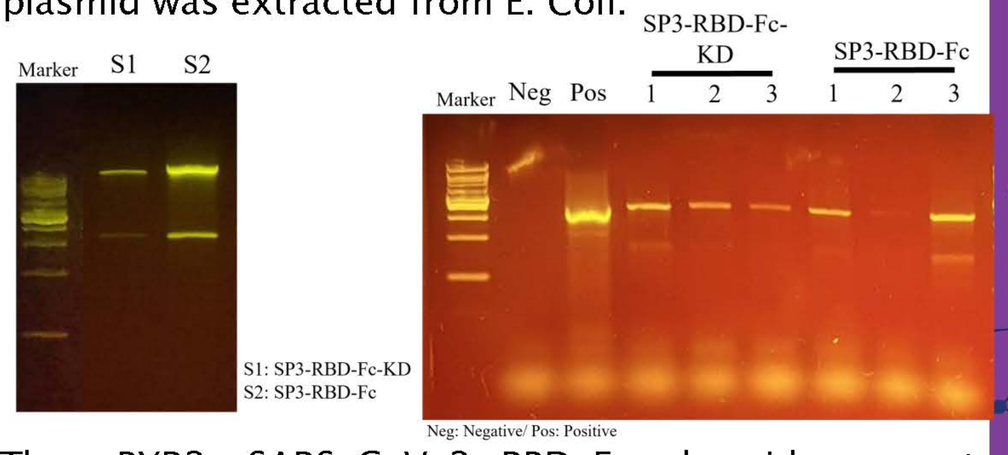
Neg: Negative/ R: Replicate

The RBD of SARS-CoV-2 (SARS-CoV-2 RBD combined with Fc region of human immunoglobulin G1 were synthesized and cloned by using the SP3 primer, SEKDEL primer and Fc primer



R: Replicate

Next, The interested gene (SP3-RBD-Fc-KD and SP3-RBD-Fc) were cut with a restriction enzyme and ligated into a geminiviral vector (pBYR2eK2Md; pBYR2e) and transferred into *E. Coli*. for increased plasmid dosage. *E. Coli* which contained pBYR2e-SARS-CoV-2 RBD-Fc vector was selected by antibiotic resistant with Kanamycin A. Then each single colony of *E. Coli* was performed PCR to select the corrected plasmid consideration of PCR product size. The plasmid was extracted from E. Coli.



S1: SP3-RBD-Fc-KD
S2: SP3-RBD-Fc

Neg: Negative/ Pos: Positive

The pBYR2e-SARS-CoV-2 RBD-Fc plasmid was cut with restriction enzyme for re-check the insertion and the plasmid was transferred into *Agrobacterium tumefaciens*. Then the colony of *A. tumefaciens* was performed PCR to select the corrected plasmid by consideration of PCR product size. The *N. benthamiana* ΔXF and wild-type plants were agro-infiltrated and harvested at 4 days post translation.

CONCLUSION

The SARS-CoV2 RBD-Fc proteins were cloned into geminiviral vector and transferred to *A. tumefaciens*. Next experiment id to protein expression in *N. benthamiana* ΔXF and wild type plants. The further experiments are Characterization of N-glycan, Mouse immunization and *In vitro* neutralization ability

ACKNOWLEGEMENT

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137], the scholarship from Chulalongkorn University, The Second Century Fund (C2F), year 2022 and Baiya Phytopharm Co., Ltd.

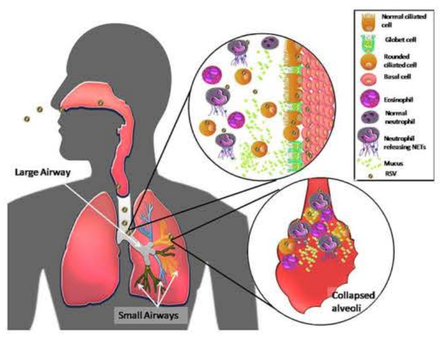
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Development of a plant-produced anti-RSV F monoclonal antibody.

Nuttapat Pisuttinusart[1,2,], Kaewta Rattanapisit[3], Chanya Srisaowakarn[4],
Arunee Thitithanyanont[4], Panithi Saktrakulkla[3] and Waranyoo Phoolcharoen[2,3,*]
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[2]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.,
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Introduction





Respiratory Syncytial Virus (RSV) is a contagious virus that can be spread through aerosols and causes lower respiratory tract infections (LTRI) in infants, children, and elderly people. In some cases, the infection causes more severe diseases and can lead to pneumonia and bronchiolitis that may require hospitalization. RSV leads to approximately 57,000 children under 5 years of age requiring hospitalization, and 100-500 deaths due to RSV infection. While approximately 177,000 elderlies above the age of 65 require hospitalization, and approximately 14,000 predicted deaths.

Currently, there is no licensed vaccine available to protect against RSV infection. However, passive immunization appears to be an option. Palivizumab, an RSV monoclonal antibody (mAb), is approved by the US Food and Drug Administration (FDA) for use prophylactically to reduce morbidity in a high-risk population of infants and children. Palivizumab clinical trials have shown reductions in the hospitalization of 45–55% compared to placebo. Nirsevimab is a recently approved monoclonal antibody used to prevent RSV infection in infants. Nirsevimab was designed to provide passive immunity against RSV by targeting the virus and neutralizing its ability to infect cells. Nirsevimab binds to RSV-F at antigenic site Ø and has been found to be 50-fold more potent than palivizumab in vitro and 9-fold more potent in vivo at equal concentrations.

Nowadays, plants can be used as a platform for producing monoclonal antibodies because they offer a cost-effective alternative for the production of therapeutic proteins. Plants can be grown in large quantities quickly and economically, resulting in a scalable manufacturing system. Plants can also perform complicated post-translational modifications on proteins, such as glycosylation, which can improve the protein function and stability. Additional benefits of plant transient expression platforms include lower risk of contamination by human pathogens, faster production times, and lower downstream processing costs.

Methodology

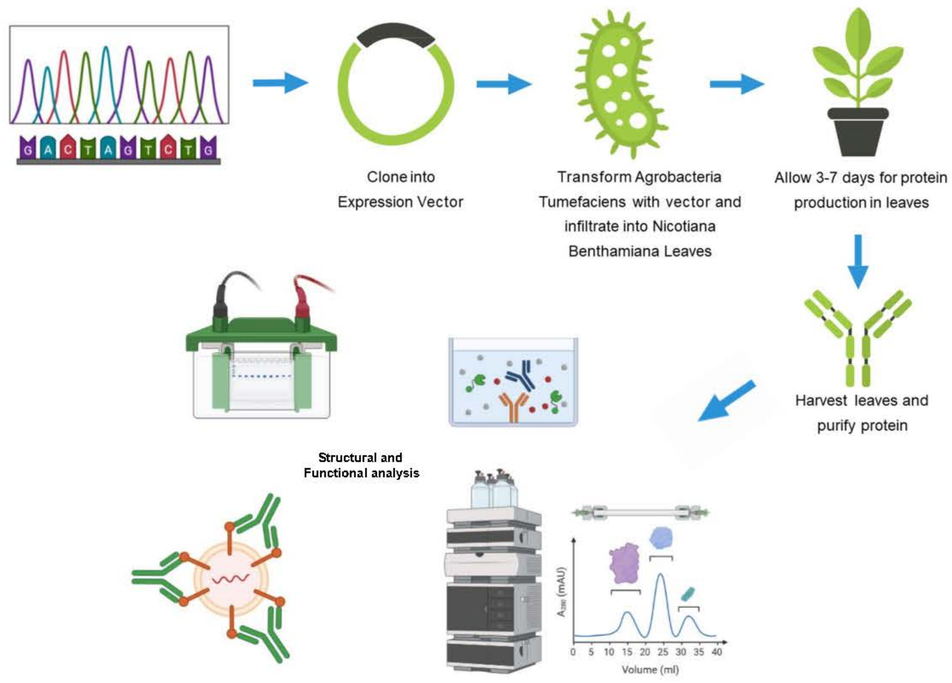
Gene designing and cloning
- Antibody name 5C4 and CR9501
- Codon optimization and gene synthesis
- Cloned into expression plasmid (germiniviral vector)

Antibody expression
- *Nicotiana Benthamiana* as expression host
- Transient expression by using agroinfiltration
- Harvest at 4-day post infiltration

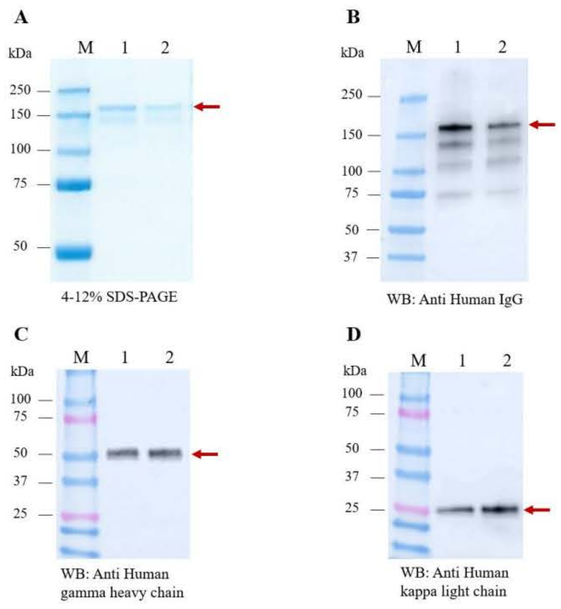
Protein extraction and purification
- Extraction using PBS in ratio 1:2 (leaf weight : Buffer)
- Crude clarify by centrifugation and filtration
- Purification using Protein A chromatography

Protein characterization
- MW determination by SDS-PAGE
- Identity by Western blot
- Purity analysis by SEC-HPLC

Functional analysis
- *In vitro* binding to RSV-F using ELISA technique
- *In vitro* neutralizing to RSV strain A2



Result



SDS–PAGE (A) and western blot (B) using anti human IgG HRP conjugated antibody of purified plant-produced anti-RSV mAbs under nonreducing conditions. For reducing condition, the membrane was detected with either HRP-conjugated anti-human gamma chain antibody (C) or anti-human kappa chain antibody (D). Lane M, Protein ladder; Lane 1, Purified plant-produced 5C4; Lane 2, Purified plant-produced CR9501, respectively.

Discussion and Conclusion

In this investigation, we have demonstrated the efficient utilization of a plant expression platform for the production of two promising anti-respiratory syncytial virus (RSV) monoclonal antibodies (mAbs), specifically 5C4 and CR9501. Both mAbs, 5C4 and CR9501, were successfully expressed in a transient manner within a short period of 4 days post-infiltration. Notably, these antibodies exhibited effective accumulation in *N. benthamiana* leaves and were amenable to purification through protein A chromatography.

However, a comprehensive investigation of the functional and physiochemical attributes of these antibodies is imperative to enhance our understanding. This in-depth analysis is pivotal for the prospective development of these antibodies, with the ultimate goal of facilitating their progression into clinical trials.

Acknowledgement

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137]

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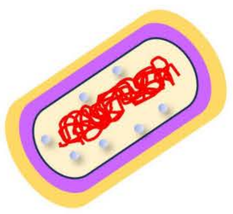
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สู่อุตสาหกรรมแห่งอนาคต

Development of Biopharmaceutical from Plant for Emerging Disease

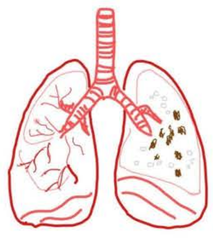
Ratama Daduang[1], Panithi Saktrakulkla[1], Waranyoo Phoolcharoen[2,3]

[1]Baiya Phytopharm Co., Ltd., Bangkok, Thailand; [2]Center of Excellence in Plant-produced Pharmaceuticals, Chulalongkorn University, Bangkok, Thailand; [3]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

INTRODUCTION



Mycobacterium tuberculosis (Mtb)



Lung infected with Tuberculosis (TB)

A significant global health threat, reporting 10.6 million new cases and 1.6 million deaths in 2021.

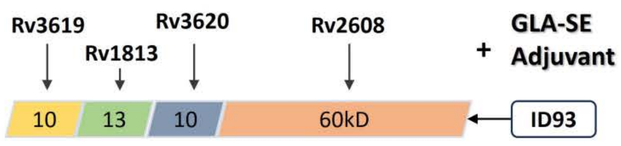


The Bacille Calmette-Guérin (BCG) vaccine, only licensed TB vaccine globally

Modest efficacy. It is most effective against severe infant TB complications, but its effectiveness in adults is limited, with inconsistent availability.

An urgent needed, more effective vaccine capable of addressing various disease stages is required.

Develop new TB vaccine candidates → **Adjuvant-containing subunit vaccine ID93 + GLA-SE showing promise.**



The safety, immunogenicity, and pharmacodynamics of the ID93 + GLA-SE vaccine have been successfully demonstrated in various animal model. Clinical studies have further affirmed the safety and immunogenicity of the ID93 + GLA-SE vaccine in diverse populations.

Plant-based Technology → **Feasible and scalable alternative platform**



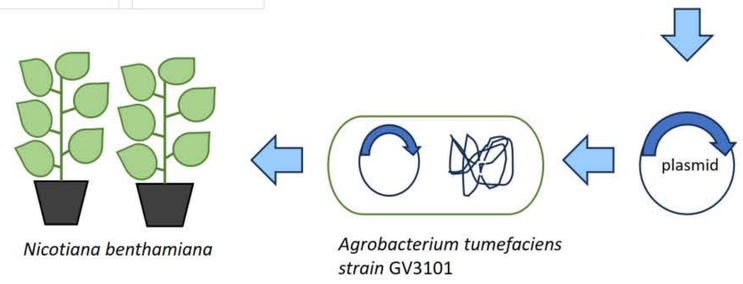
Develop low-cost, effective vaccine candidate

The study focuses on constructing, producing, and characterizing TB vaccine candidates in plants.

METHODS

Rv3619	Rv1813
Rv3620	Rv2608

→ **Commercially synthesized with codon optimization**



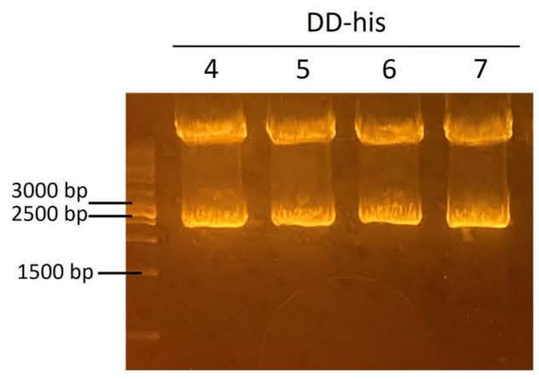
RESULTS AND DISCUSSION

Genes synthesis and Codon optimization

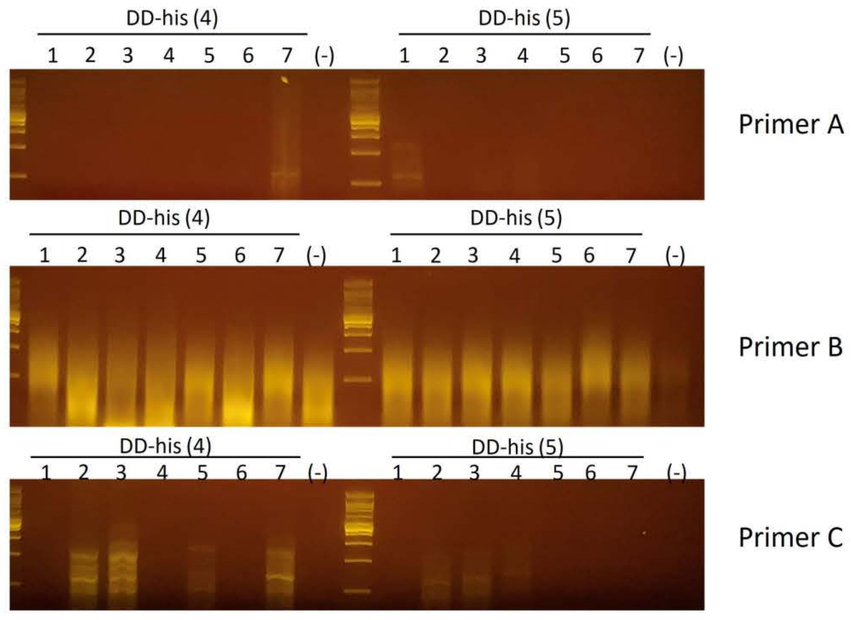
- **Target genes' region was designed and commercially synthesized with codon optimization.**

Plasmid construction

- **DD-his (target genes with his-tag) was successfully constructed and transformed to Agrobacterium GV3101.**



The plasmid pUC harbored DD-his was verified through restriction enzyme digestion.



The presence of DD-his in Agrobacterium was confirmed using colony-PCR with 3 sets of primers.

CONCLUSION

- **The target gene region was successfully constructed and transformed to *Agrobacterium tumefaciens* strain GV3101.**
- **The target clones will be verified by sequencing before being transformed to *Nicotiana benthamiana*.**

ACKNOWLEDGEMENT

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137]

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Development of hydrogel prototype for steroid drug delivery system from Thai Silk Fibroin and study efficiency of Thai Silk Fibroin hydrogel for prolong steroid drug release

Kriangsaksri R.[a], Keeratihattayakorn S.[a,b], and Ratanavaraporn J.[a,b,*]

[a] EngineLife co.,ltd.

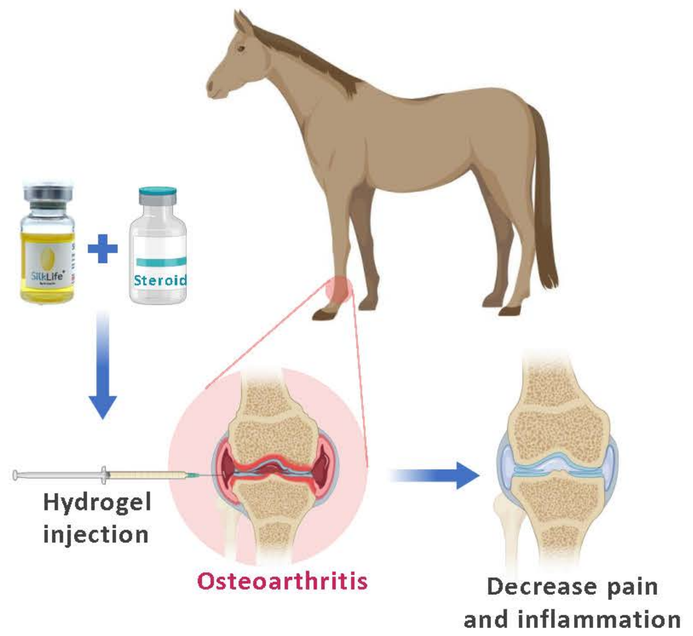
[b] Biomedical Engineering Program, Biomedical Engineering Research Center, Faculty of Engineering, Chulalongkorn University

* **Corresponding author:** Juthamas.R@chula.ac.th

Introduction

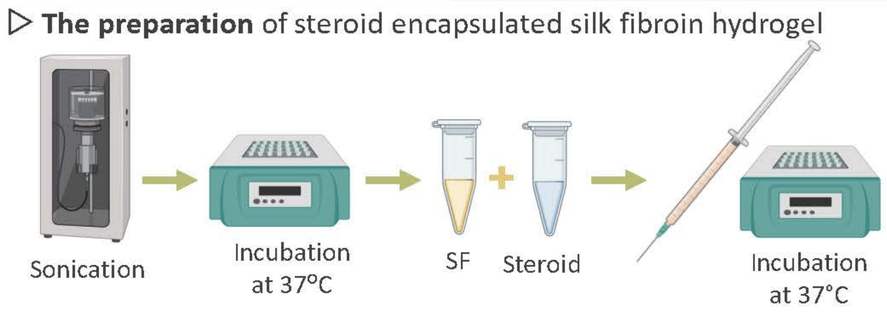
Osteoarthritis (OA) is a leading cause of lameness in horses, characterized by pain, stiffness, and swelling resulting from the breakdown of cartilage between bones. While steroids are commonly used for OA treatment, their rapid degradation due to acid hydrolysis by uric acid within the horse's joint can lead to the need for repeated doses and potential overdose.

This research investigates the use of silk fibroin hydrogel as a drug delivery system to effectively slow and extend the release of steroids. The inclusion of steroids within silk fibroin hydrogel holds the potential to enhance treatment efficacy, reducing the necessity for repeated injections. Additionally, silk fibroin hydrogel, derived from natural **silk fibroin solution (SF)**, exhibits biocompatibility, biodegradability, and non-immunogenicity, ensuring complete degradation within the body. Its injectability through a needle, coupled with controlled drug release, aids in prolonging drug effectiveness, minimizing the need for repeat injections, and mitigating potential drug side effects.



Methods

▷ **The preparation** of steroid encapsulated silk fibroin hydrogel



▷ **The injection stability test**

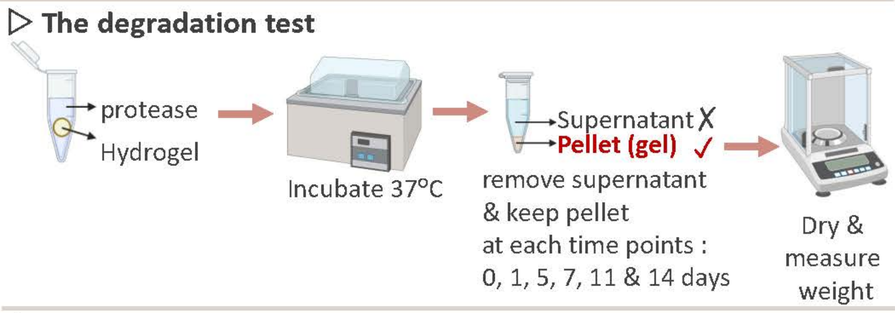
Steroid-SF hydrogel with different SF concentration were prepared :

- 0.5% w/v SF
- 1.0% w/v SF
- 2.0% w/v SF

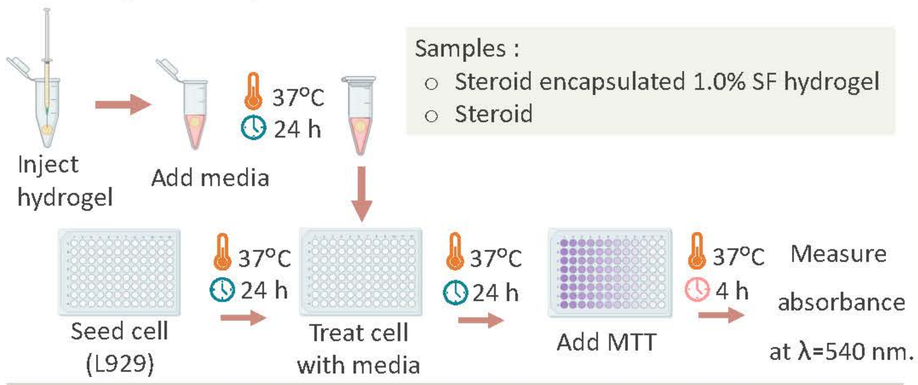


Injection samples at each time points : 0, 1, 7 & 14 days

▷ **The degradation test**



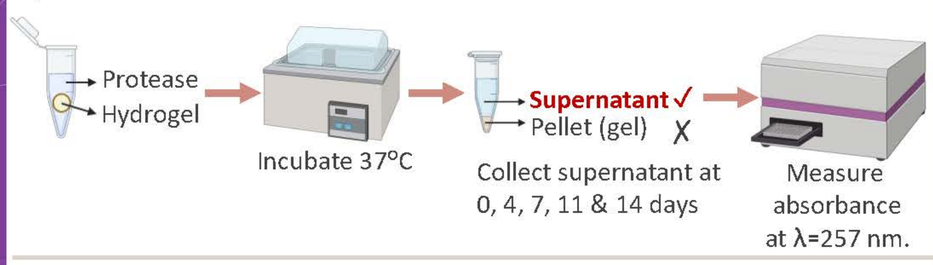
▷ **The cytotoxicity test**



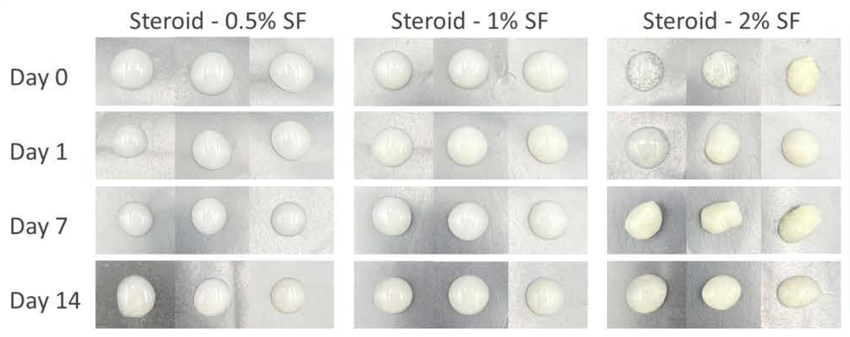
Samples :

- Steroid encapsulated 1.0% SF hydrogel
- Steroid

▷ ***In vitro* release**



Results & Discussion



" The injection of 0.5, 1.0 and 2.0% SF hydrogel, 0.5% and 1.0% SF hydrogel could be passed though the needle while 2.0% SF hydrogel occur gel clogged inside needle and separate between gel and water. "

Figure 1

The injection stability of steroid encapsulated in 0.5, 1.0 & 2.0% SF hydrogel at 0, 1, 7 & 14 days.

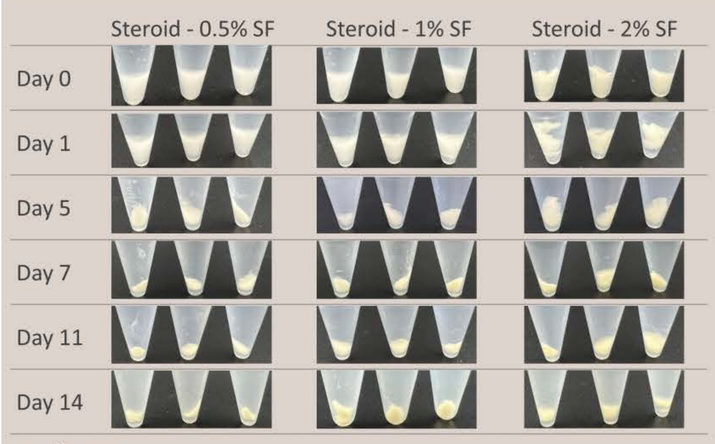


Figure 2

Degradation of steroid-SF hydrogel from 0.5, 1.0 & 2.0% SF after incubate with protease at 37°C for 0, 1, 5, 7, 11 & 14 days.

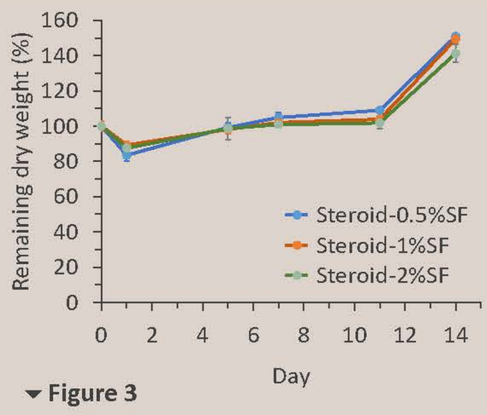


Figure 3

Percent remaining weight of steroid-SF hydrogel from 0.5, 1.0 & 2.0% SF after incubate with protease at 37°C for 0, 1, 5, 7, 11 & 14 days.

" From figure 2 show all hydrogels decrease after incubation with protease from 0 to 14 days. But figure 3 show all hydrogels weight were not different, the weight drop in 1 day and increase from 5 to 14 days that might be affected from enzyme denature and sediment. "

Figure 4

Percentage of cell viability after treat with DMEM media (negative control), media incubate with steroid-1%SF hydrogel, steroid and 20 ppm zinc acetate (positive control).

"The steroid-1%SF hydrogel demonstrated non-toxicity to cells, exhibiting a high cell viability rate of 72.87%. Conversely, the steroid alone showed an impact on cell viability and induced cellular toxicity."

Figure 5

Percentage of steroid cumulative *in vitro* release from steroid and steroid-1%SF hydrogel after incubate at 37°C for 0, 4, 7 & 14 days.

" Steroid remaining in joint fluid after injection for 14 days while steroid from hydrogel was slow release for 14 and some steroid still entrap inside hydrogel "

Conclusion

The steroid encapsulated 1.0% SF hydrogel was chosen because it has smooth texture than 0.5% and 2.0% SF and 2.0%SF hydrogel injection would be clogged in needle. Moreover 1.0% SF will promote slower release than 0.5% SF because it contains with higher protein concentration and might be slow degraded. The steroid encapsulated 1.0% SF hydrogel indicate biocompatible property from low toxicity result that also protected cell from steroid drug effect and show prolong release efficiency in both *in vitro* and *in vivo* experiment.

Acknowledgements

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] and The Second Century Fund (C2F), High Impact Program, Chulalongkorn University for providing the financial support.

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Development of a delivery system for the controlled release of Platelet-Rich Plasma (PRP) from Thai silk fibroin

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[a] EngineLife co., ltd.

[b] Biomedical Engineering Program, Biomedical Engineering Research Center, Faculty of Engineering, Chulalongkorn University

* **Corresponding author:** Juthamas.R@chula.ac.th

Introduction

Osteoarthritis (OA) stands as the predominant chronic joint ailment causing physical impairment. Traditional drug therapies often prove ineffective in halting disease progression. **The platelet-rich plasma (PRP)** emerges as a promising alternative for regenerating and maintaining articular cartilage in OA.

Silk fibroin (SF), a versatile biomaterial, has found diverse applications in medicine due to its unique properties. Notably, silk fibroin's popularity has surged as a delivery system, offering structural and functional support while mitigating associated side effects.

This study aims to assess the efficacy of a silk fibroin-encapsulated PRP hydrogel for treating OA. The controlled release of PRP by the SF hydrogel within joints aims to prolong PRP presence compared to conventional methods. **This approach holds the potential to amplify treatment effectiveness, minimize dosage requirements, and reduce injection frequency.**

On administration of PRP/SF hydrogel therapeutics into the fetlock joints:

- **Right:** PRP
- **Left:** PRP/SF hydrogel

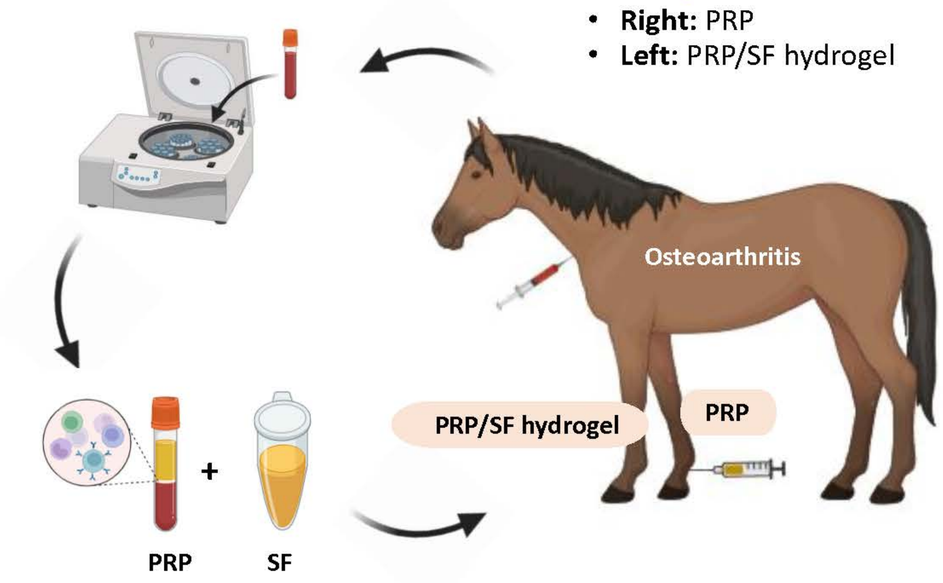
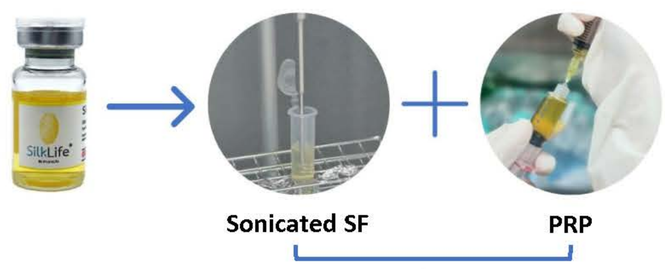


Fig. 1. Administration of PRP/SF hydrogel injection for OA treatment.

Methods

Preparation of PRP/SF Hydrogel Injection

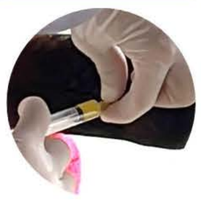


TGA



- **SF solution**
- **PRP**
- **PRP/SF hydrogel**

***In vivo* in horse**



- **PRP: right fetlock joint**
- **PRP/SF hydrogel: Left fetlock joint**

Results & Discussion

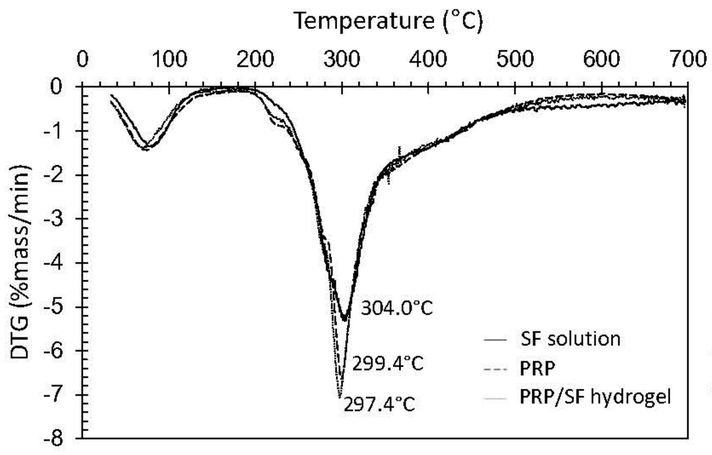


Fig. 2. Thermogravimetric analysis (TGA) of SF solution, PRP, and PRP/SF hydrogel.

TGA revealed that the PRP/SF hydrogel exhibited interactions between the SF solution and PRP. Although there was **no significant difference** in the decomposition temperature of PRP and PRP/SF hydrogel (Fig. 2).

Table 1 Quantification of **white blood cell (WBC)** count and **total protein (TP)** levels in PRP and synovial fluid before and after PRP/SF hydrogel injection.

Horse	PRP (Day 0)		Synovial fluid (14 days)			
	WBC (10^3 cells/µL)	TP (g/dL)	WBC (10^3 cells/µL)		TP (g/dL)	
			Right	Left	Right	Left
P01	0.03	6.3	0	0	0.8	2.2
P02	0.03	6.6	0	0	1.0	2.5
P03	0.01	5.3	0	0	0.5	1.3
P04	0.03	5.3	0	0.01	0.7	1.5
P05	0.03	6.1	0	0	0.7	2.4
P06	0.99	7.2	0	0.01	0.7	0.6
P07	5.62	6.6	0	0	0.6	1.1
P08	0.01	5.6	0	0	0.7	1.1
P09	0.06	5.2	0	0	0.5	0.5

* PRP injected in the right joint and PRP/SF hydrogel injected in the left joint

After PRP/SF hydrogel injection, **no inflammation or sensitization signs** were observed in horses. WBC count and physical examination at 14 days showed no issues. Total protein levels in the left joint were higher than the right, attributed to silk fibroin hydrogel (Table 1).

Table 2 The total platelet count in synovial fluid at 14 days.

Sample	% Total Platelet
PRP/SF hydrogel	3.66 ± 5.37[a]
PRP	1.68 ± 2.90[b]

*Paired Samples T-test, One-sided *p-value* = 0.042

The comparison of the total platelet count between PRP/SF hydrogel and PRP showed that SF hydrogel can maintain quality and control the release of PRP (Table 2).

Conclusions

- The SF solution interacts with PRP.
- The PRP/SF hydrogel can more effectively protect against the degradation of PRP than the injection of PRP alone.
- The results show great potential for future use in the treatment of osteoarthritis.

Acknowledgements

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] and The Second Century Fund (C2F), High Impact Program, Chulalongkorn University for providing the financial support.

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Development of Artificial Diet Suitable for Silkworm Growth and Cocoon Production for Use in Medical Applications

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[a] EngineLife co.,ltd.

[b] Biomedical Engineering Program, Biomedical Engineering Research Center, Faculty of Engineering, Chulalongkorn University

* **Corresponding author:** Saran.ke@chula.ac.th

Introduction

Silkworms (*Bombyx mori* L.)



Fresh mulberry leaves

Seasonal Variations in Silkworm Rearing
Influence of Mulberry Nutrient Content on Cocoon Growth and Production



Cannot be cultivated year-round, leading to seasonal limitations in silkworm rearing.

Control

Artificial Diet

Enhancing Silkworm Growth and Cocoon Quality
A Nutrient-Supplemented Artificial Diet with Dried Mulberry Powder



- ❖ **Composed of dried mulberry powder and supplemented with various nutrients**
- ❖ **Custom-made to optimize silkworm growth**
- ❖ **Enables year-round silkworm rearing**
- ❖ **Higher-quality cocoons**

Methods

Sericulture Model Chamber



26±2°C and relative humidity maintained at 69±7%.

Group A
High Protein (45.44%) and Low Carbohydrate (8.08%)

Formulation and Preparation of an Artificial Diet is show in **Fig.1.**

Group B
Low Protein (20.44%) and High Carbohydrate (33.08%)



Artificial Diet

Effect of Diet on Silkworm Larvae Rearing

During the transition from the 4th instar to the 5th instar, the larvae were provided with their diet.
The diet options included fresh mulberry leaves (**Control**) or an artificial diet, categorized as **Group A** and **Group B**.



Ingredient dry powder (mulberry leaves powder, tapioca starch, soybean meal and other ingredient) + Ingredient liquid (water, agar, mineral and other ingredient) → Mix liquid and dry ingredients → Steam the mixture → Artificial Diet for silkworm

Fig. 1. Formulation and Preparation of an Artificial Diet:
A Modification of the Method Proposed by **Chotimanothum B., et al. (2019)[1].**

Results & Discussion

Silkworm survival rate

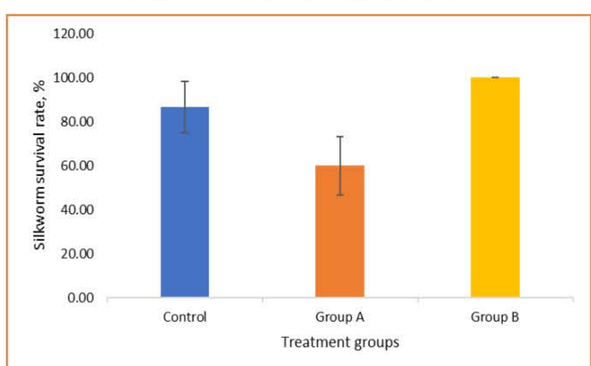


Fig. 2. Silkworm survival rate for the three group.

$$Silkworm\ survival\ rate\ \% = \frac{No.\ of\ silkworm\ survival}{Total\ No.of\ larvae\ reared} \times 100$$

Cocoon production rate

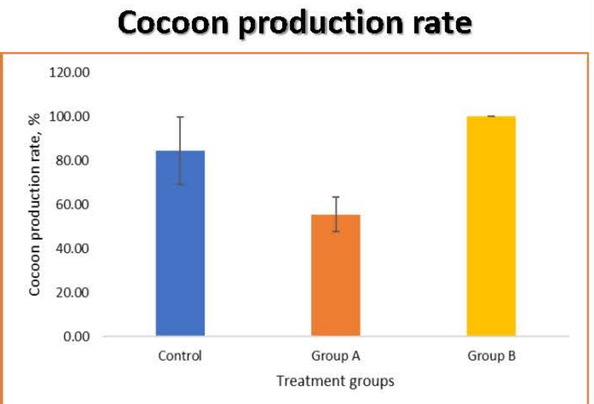


Fig. 3. Cocoon production rate for the three group.

$$Cocoon\ production\ rate\ \% = \frac{No.\ of cocoon\ harvested}{Total\ No.of\ larvae\ reared} \times 100$$

The artificial diet (**Group B**) had a higher survival rate and cocoon production rate than the **control group** and **Group A**

Silkworms (4th instar to the 5th instar) need carbohydrates more than protein[2], they have a high survival rate in **Group B**. Carbohydrates are the main source of energy for most insects[3]. Therefore, it is expected that carbohydrates are a very important source of energy for spinning silk threads for silkworms. This resulted in a high cocoon shell ratio in **Group B.**

Cocoon shell ratio

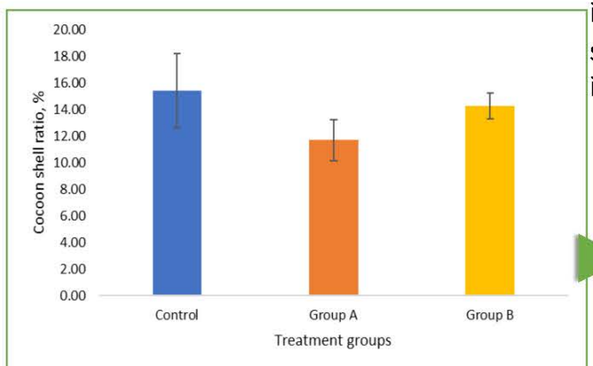


Fig. 4. Cocoon shell ratio for the three group.

$$Cocoon\ shell\ ratio\ \% = \frac{Weight\ of\ cocoon\ shell}{Weight\ of\ cocoon} \times 100$$

The cocoon shell ratio in artificial food (**Group B**) was slightly lower compared to **the control group**.

Nutrition quality

Table 1. Nutrition quality of fresh mulberry leaves, mulberry leaf powder and artificial diet (**Group A**).

Test item	Unit	Result		
		Fresh mulberry leaves	Mulberry leaves powder	Artificial diet for silkworm (Group A)
Ash	g/100g	5.56	14.34	2.48
Calories	Kcal/100g	108.73	375.14	96.50
Calories from Fat	Kcal/100g	11.61	74.34	8.46
Carbohydrate	g/100g	18.60	60.04	14.23
Fat	g/100g	1.29	8.26	0.94
Moisture	g/100g	68.87	2.20	74.57
Protein (%N x 6.25)	g/100g	5.68	15.16	7.78
Total Dietary Fiber	g/100g	16.79	27.38	8.97

The artificial diet (**Group A**) had a higher protein content than fresh mulberry leaves. On the other hand, fresh mulberry leaves had higher carbohydrate **content group** than the artificial diet (**Group A**).

Conclusion

The artificial diet formula (Group B) had higher survival and nesting rates compared to the control group. The artificial diet was suitable for the growth of silkworms, causing the production of silk cocoons to be similar to the control group.

A further plan is to improve the artificial formula by adding silver nanoparticles. Then the chemical properties and mechanical properties of the cocoons were tested.

Acknowledgements

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] and The Second Century Fund (C2F), High Impact Program, Chulalongkorn University for providing the financial support.

Reference

[1] Banthari Chotimanothum et.al. (2019). Diet Formular Development for Silkworm Rearing (*Bombyx mori* L.). รายงานผลการวิจัยหม่อนไหม 2563. 318-336.

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Development of GRAFTED COPOLYMER THAI SILK FIBROIN for *In Situ* Injectable Hydrogel, as Drug Delivery Systems

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* **Corresponding author:** Juthamas.R@chula.ac.th

Introduction

Thai silk fibroin (SF)

has shown immense potential as **a drug delivery carrier and as scaffolds for tissue engineering**





SF properties

- ❖ Approved biodegradable polymer
- ❖ Highly biocompatible
- ❖ Highly mechanical properties

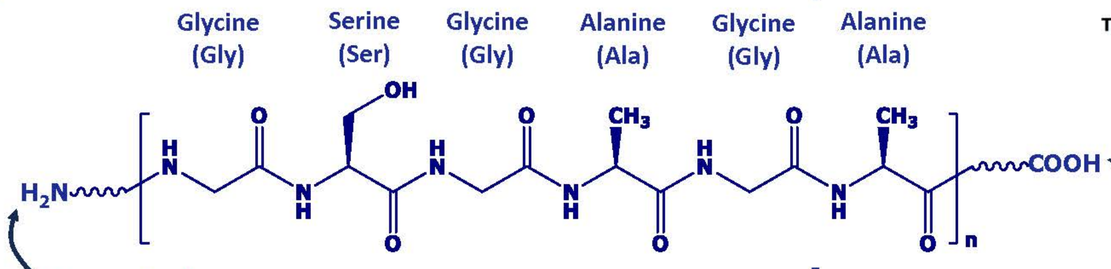
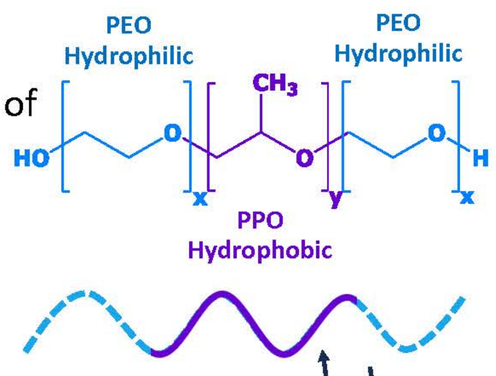


Fig. 1. Chemical structure of SF.

Poloxamer (PLX)

Nonionic triblock copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO)



PLX properties

- ❖ Thermo-reversible hydrogel
  - ❖ Exhibit low toxicity
  - ❖ Water soluble

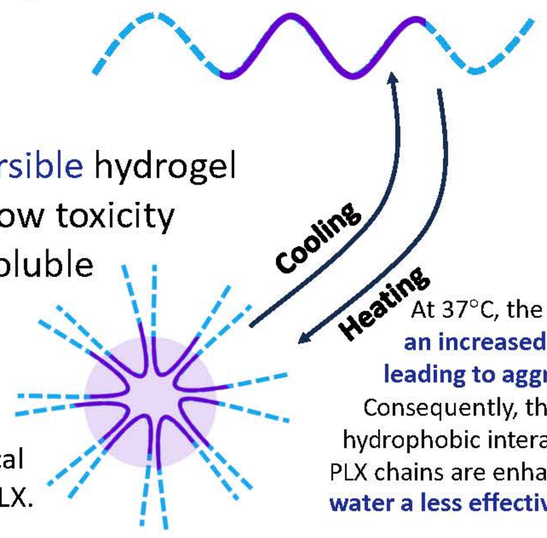
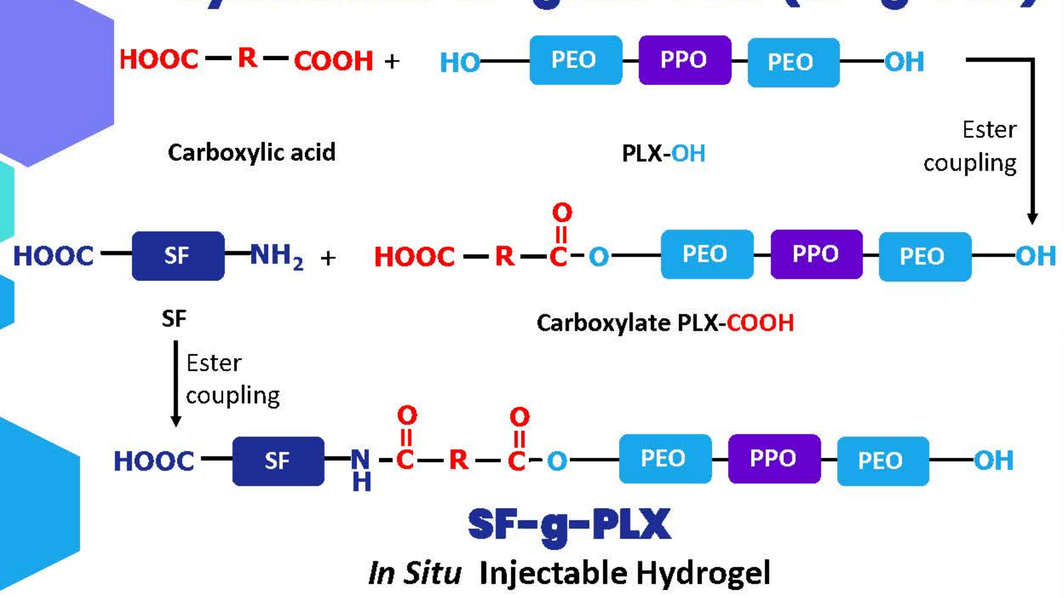


Fig. 2. Chemical structure of PLX.

At 37°C, the PPO blocks exhibit **an increased hydrophobicity, leading to aggregation.** Consequently, the intermolecular hydrophobic interactions between PLX chains are enhanced, **rendering water a less effective solvent.**

Methods

Synthesize SF-graft-PLX (SF-g-PLX)



SF-g-PLX

In Situ **Injectable Hydrogel**

DEFINITION:

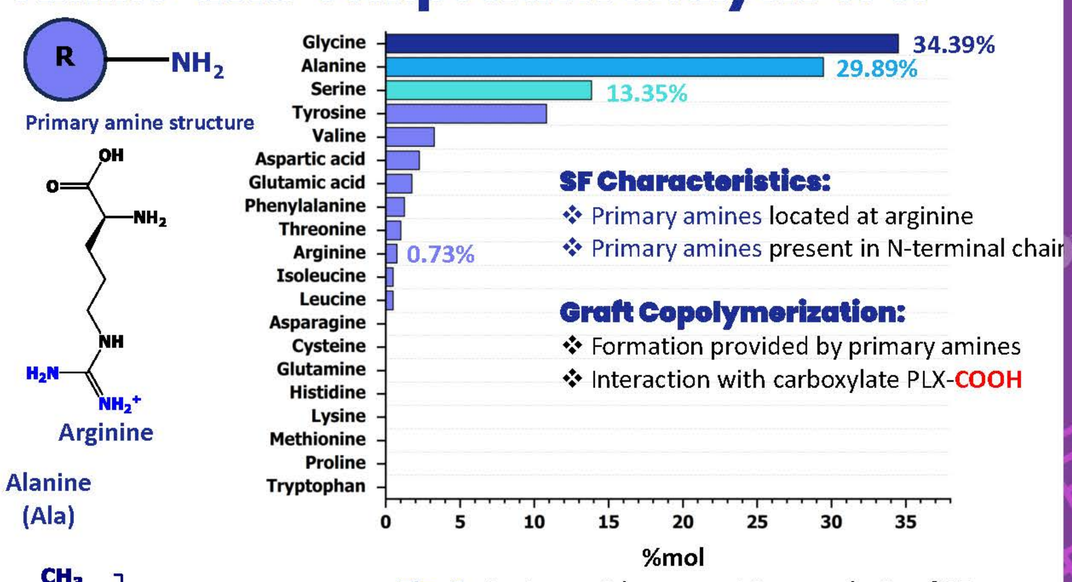
- ❖ A hydrogel that transforms from liquid to gel
- ❖ Injectable into the body
- ❖ Formation of a gel-like structure at the target site

NEW PROPERTIES

- ❖ **Enhanced** mechanical properties
- ❖ **Improved** biocompatibility
- ❖ **Enhanced** drug release rates
- ❖ **Lower** cytotoxicity

Results & Discussion

Amino acid composition analysis of SF



SF Characteristics:

- ❖ Primary amines located at arginine
- ❖ Primary amines present in N-terminal chain

Graft Copolymerization:

- ❖ Formation provided by primary amines
- ❖ Interaction with carboxylate PLX-COOH

Fig. 3. Amino acid composition analysis of SF.

Gelation Time

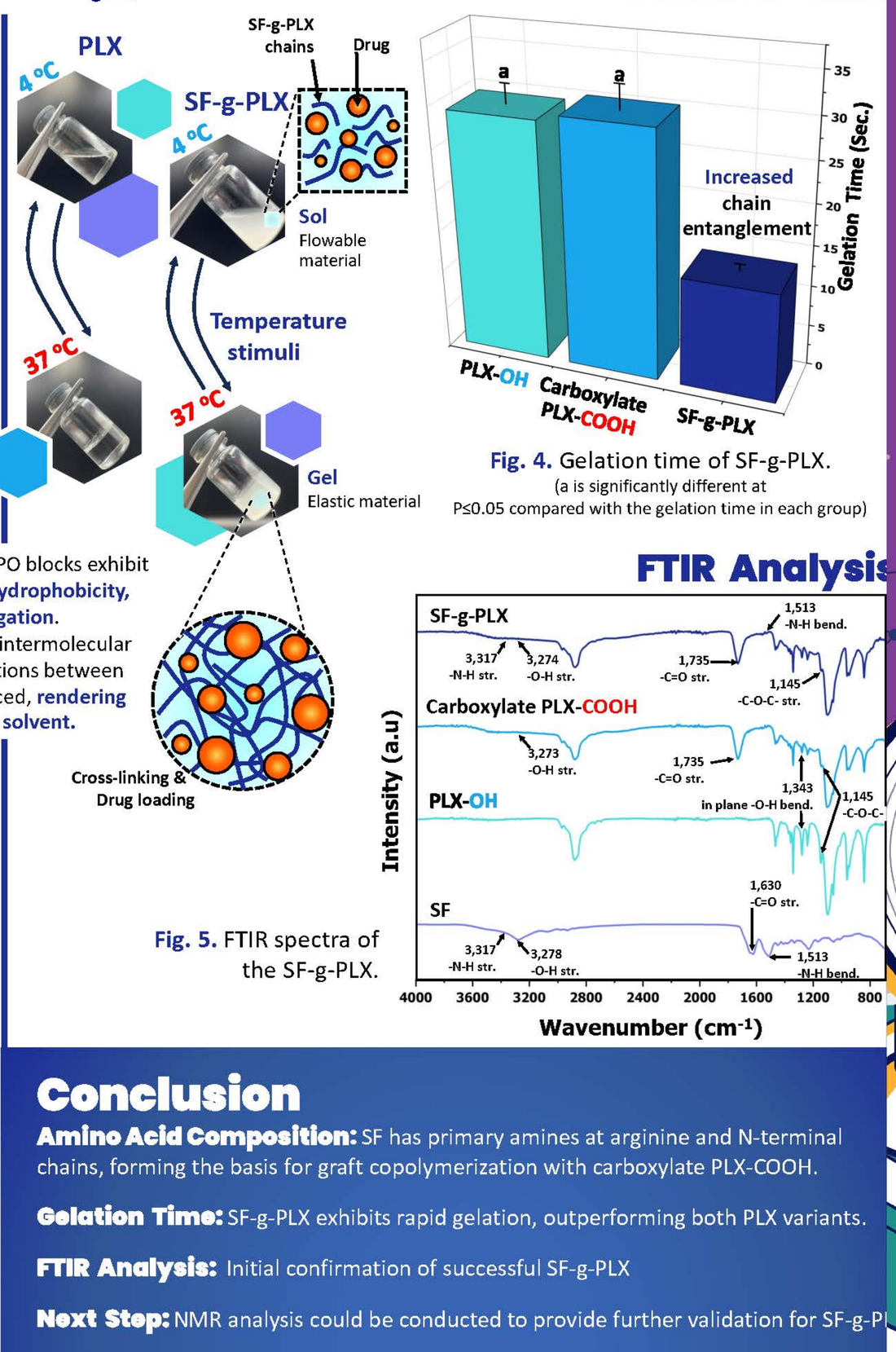


Fig. 4. Gelation time of SF-g-PLX.
(a is significantly different at P≤0.05 compared with the gelation time in each group)

FTIR Analysis

Fig. 5. FTIR spectra of the SF-g-PLX.

Conclusion

Amino Acid Composition: SF has primary amines at arginine and N-terminal chains, forming the basis for graft copolymerization with carboxylate PLX-COOH.

Gelation Time: SF-g-PLX exhibits rapid gelation, outperforming both PLX variants.

FTIR Analysis: Initial confirmation of successful SF-g-PLX

Next Step: NMR analysis could be conducted to provide further validation for SF-g-PL

Expected outcome: This research aims to present an injectable biomaterial for tiss engineering and drug delivery applications.

Acknowledgements

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Development of A Controlled Drug Delivery System from Thai Silk Protein-Based Biomaterials for Medical Applications

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[b] Biomedical Engineering Program, Biomedical Engineering Research Center, Faculty of Engineering, Chulalongkorn University

* **Corresponding author:** Slila.chayanun@gmail.com

Introduction

Mupirocin Hydrogel:

A fibroin-based formulation with mupirocin for enhanced wound healing, specifically targeting bacterial pathogens that often hinder skin wound healing.

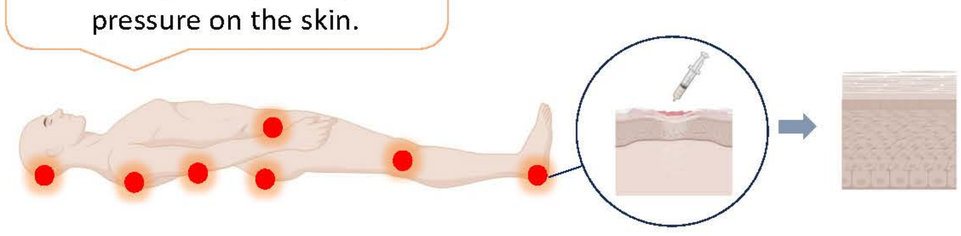
Targeted Application

Highly effective for treating deep and slow-healing wounds, including pressure sores and diabetic ulcers.

Mechanisms of Action

- Maintains a conducive, moist environment for optimal wound healing.
- Facilitates the natural process of dead tissue removal.
- Ensures a controlled release of mupirocin, effectively preventing bacterial infections in wounds.

Pressure ulcers are injuries to skin and underlying tissue resulting from prolonged pressure on the skin.



Methods



Results & Discussion

Table 1 Antimicrobial activity (R) of different mupirocin concentration on *Pseudomonas aeruginosa.*

Hydrogel	Antimicrobial activity (R)
SF hydrogel (Control)	0.07
SF hydrogel + 0.5%w/v Mu	1.25
SF hydrogel + 1.0%w/v Mu	3.51
SF hydrogel + 1.5%w/v Mu	4.17
SF hydrogel + 2.0%w/v Mu	**5.10**

***Antimicrobial activity (R) > 5.0 = Antimicrobial effectiveness**

The increased concentration of mupirocin leads to an increase in the Antimicrobial activity (R) value.

Results & Discussion

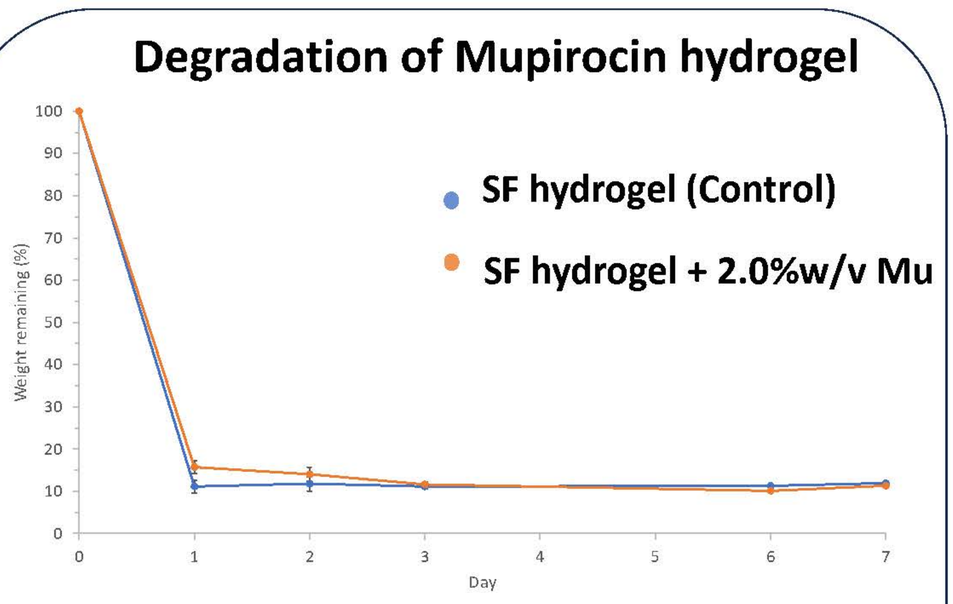


Fig. 1. Degradation behavior of Mu-loaded SF Hydrogel.

A significant weight loss (90 wt%) is observed within 1 day, which then gradually stabilizes.

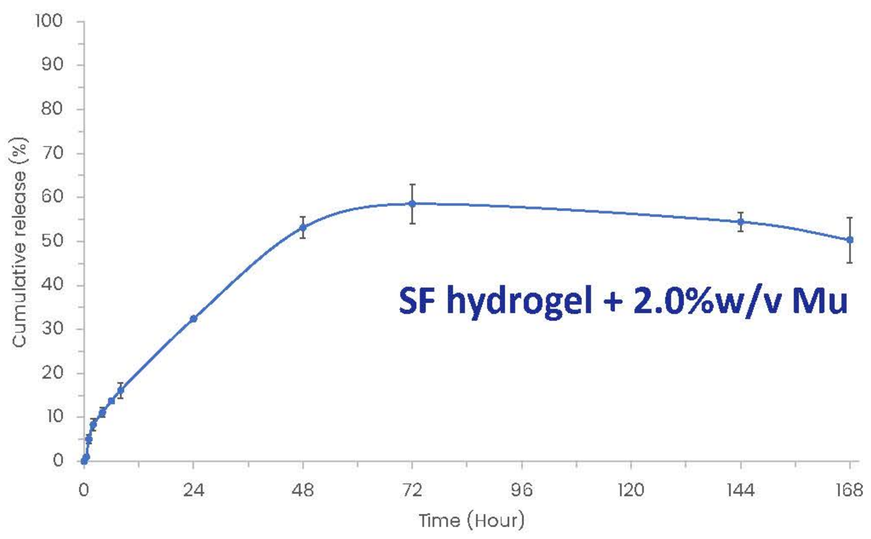


Fig. 2. Cumulative release behavior of MU loaded into SF Hydrogel.

Initial high release rate within 48 hours, followed by a steady rate from 48 to 168 hours.

Conclusion

- **Antimicrobial activity:** Highly antimicrobial effectiveness at 2%w/v Mu concentration
- **MU released:** Prolonged release for up to 168 hours
- **Expected outcome:** An option to demonstrate the choice of treatment for enhanced wound healing, specifically targeting bacterial pathogens that often hinder skin wound healing

Acknowledgements

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Thai silk patch for transdermal delivery and controlled release of Cannabis extract

Prakobkaew N.[a], Ratanavaraporn J.[a,b], Keeratihattayakorn S.[a,b] and Siritientong T.[c,*]

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b Biomedical Engineering Program, Biomedical Engineering Research Center, Faculty of Engineering, Chulalongkorn University
c Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University.
*Corresponding author: tippawan.s@pharm.chula.ac.th

Introduction



Thai silk fibroin (SF) has been potential for use in biomaterial applications like cosmetics, food, membranes, controlled release carriers, pharmaceutical stabilizers, and scaffolds.

Cannabidiol (CBD) is a non-psychoactive compound derived from the cannabis plant. Unlike tetrahydrocannabinol (THC), CBD does not induce psychoactive effects. It has been suggested that CBD may aid in alleviating insomnia by mitigating factors such as anxiety and pain that can disrupt sleep.

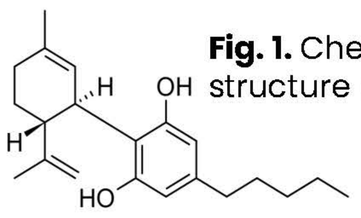
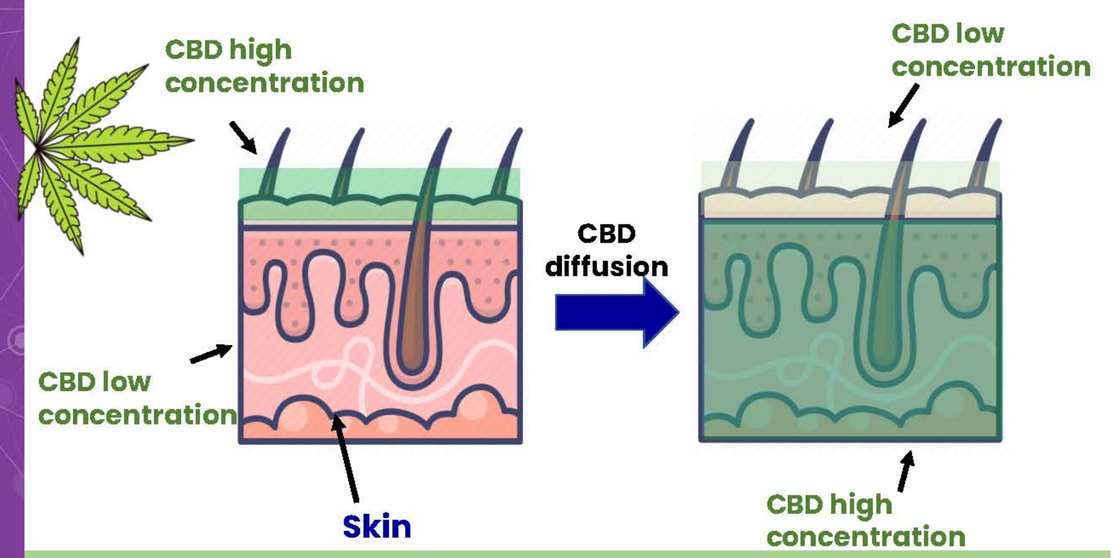


Fig. 1. Chemical structure of CBD.





Methods

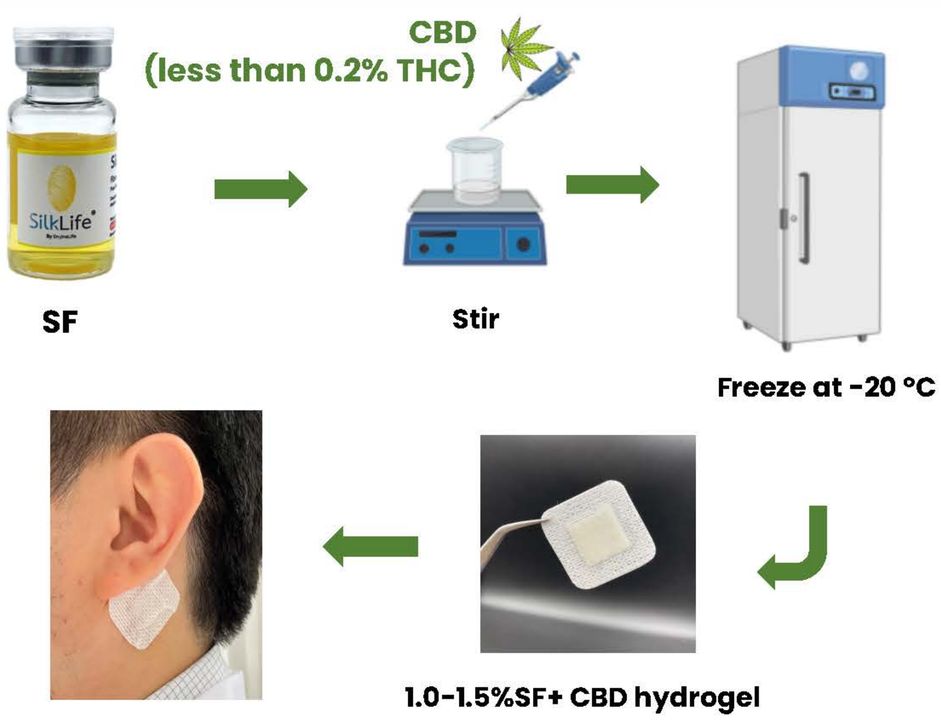


Fig. 2. Scheme of preparation of CBD patch.

Conclusion

- The 1.0%SF+CBD hydrogel showed a higher swelling degree than the 1.5% SF+CBD hydrogel due to the effect of entanglement
- The release profile showed that the silk patch released 100% of CBD in 4-8 h.
- The market research presented the users are quite satisfied especially chronic insomnia

Results & Discussion

Swelling Degree

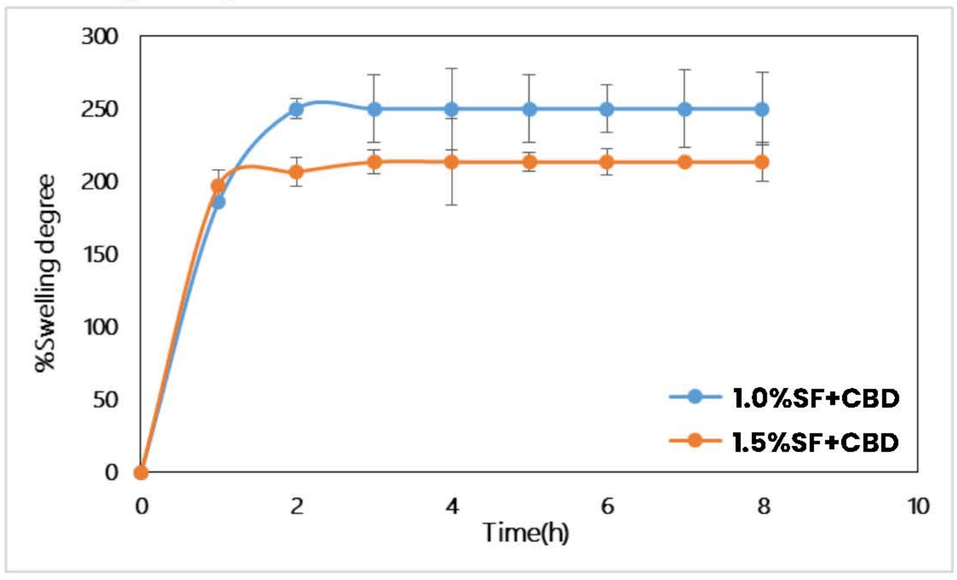


Fig. 3. The swelling degree of hydrogel patch.

The 1.5%SF+CBD hydrogel showed **higher chain entanglement** than the 1.0%SF+CBD hydrogel due to the swell degree of the 1.5% SF+CBD is less than the 1%SF+CBD.

Release Profile

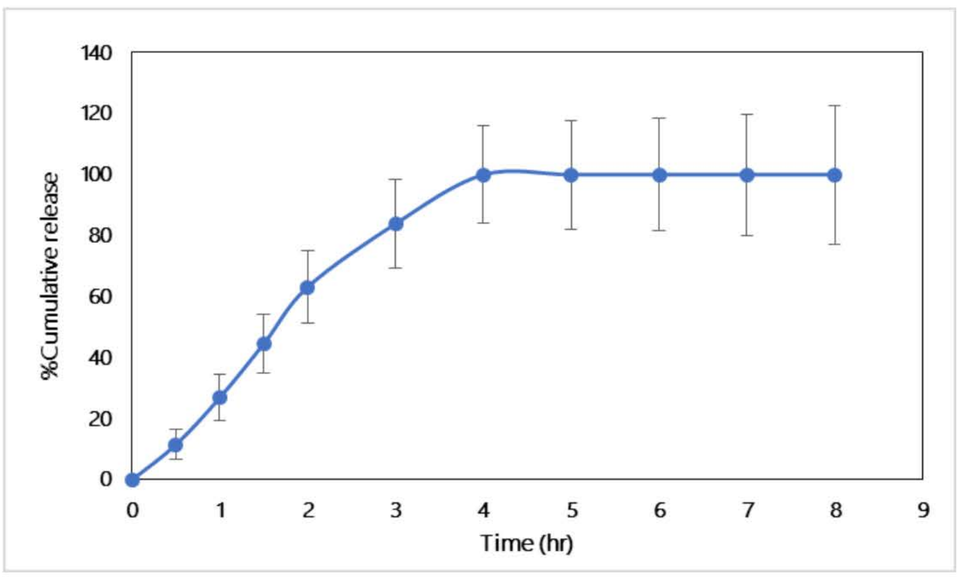


Fig. 4. Release profile of 1.0%SF+CBD hydrogel.

The cumulative release rate of the hydrogel patch was **more than 60% of the total CBD loading from initial release to 2 h**, and about 100% of the hydrogel patch was released cumulatively for 4- 8 h.

Market Research



Acknowledgements

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Development of Cosmeceuticals Product Prototype from Sericin

Kowwilaisang P.[a], Ratanavaraporna J.[a,b], Keeratihattayakorn S.[a,b] and Siritientongc T.[c,*]

[a] EngineLife co.,ltd.
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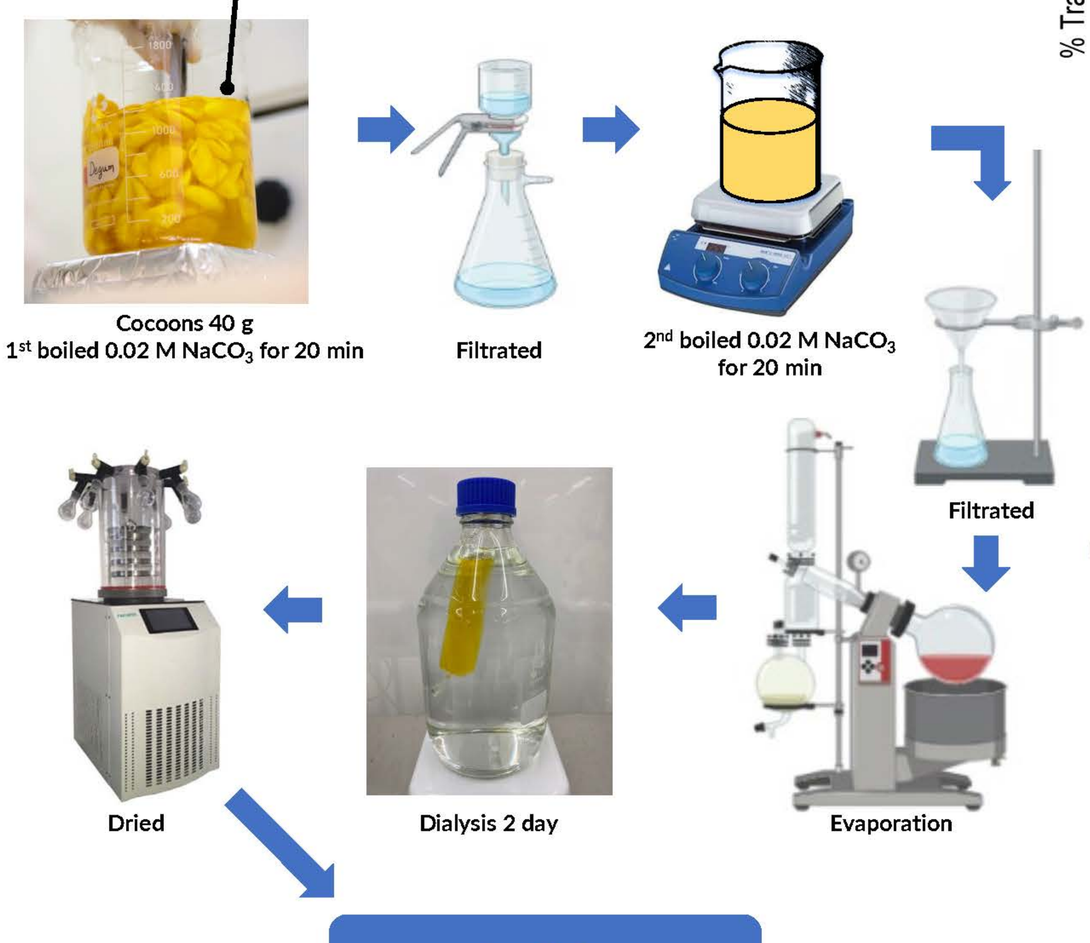
Introduction



Sericin, a gummy protein, plays a crucial role in binding the fibroin fibers together within the cocoon, acting as a natural adhesive. While sericin contains amino acids and peptides known for their robust antioxidant activity, it is often removed during the silk production process. However, its potential benefits, particularly in mitigating oxidative stress and protecting cells from damage, **have been subjects of study due to its antioxidant properties.**

Methods

1. Silk degumming batch process



Results & Discussion

1. ABTS radical scavenging activity

Table 1. Antioxidant activity of sericin protein by ABTS assay.

Samples	Ic_{50} values (µg/mL)
Ascorbic Acid	0.012
TT	**0.285**
NN	0.587
LS	0.497

TT demonstrated significantly higher $ABTS^{\bullet+}$ scavenging activity compared to NN and LS ($p < 0.05$).

2. FTIR

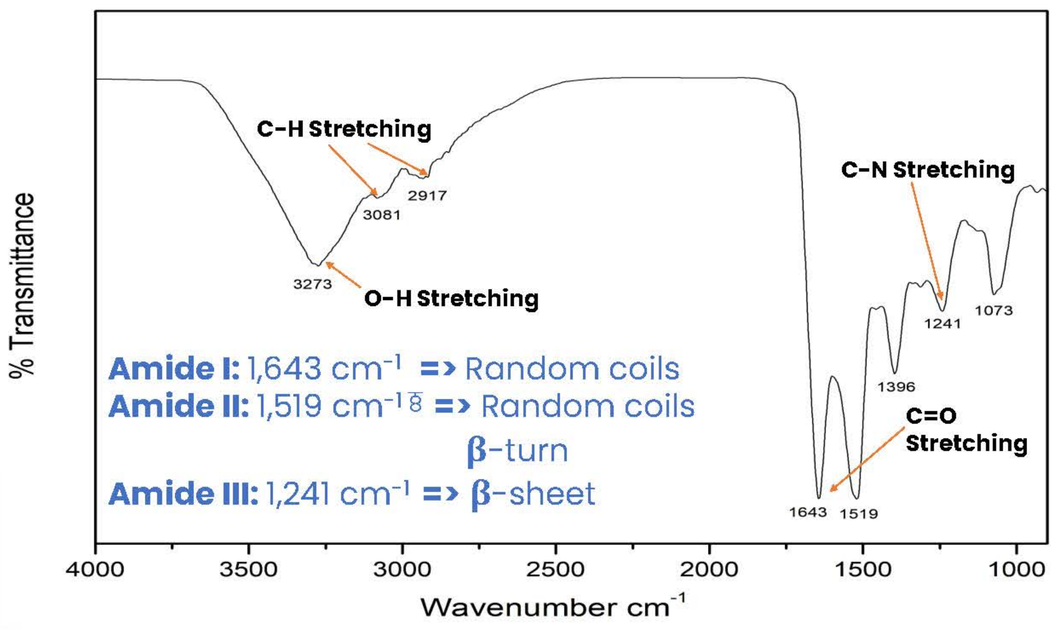


Fig.1. FT-IR spectra of the Sericin extract.

3. DSC

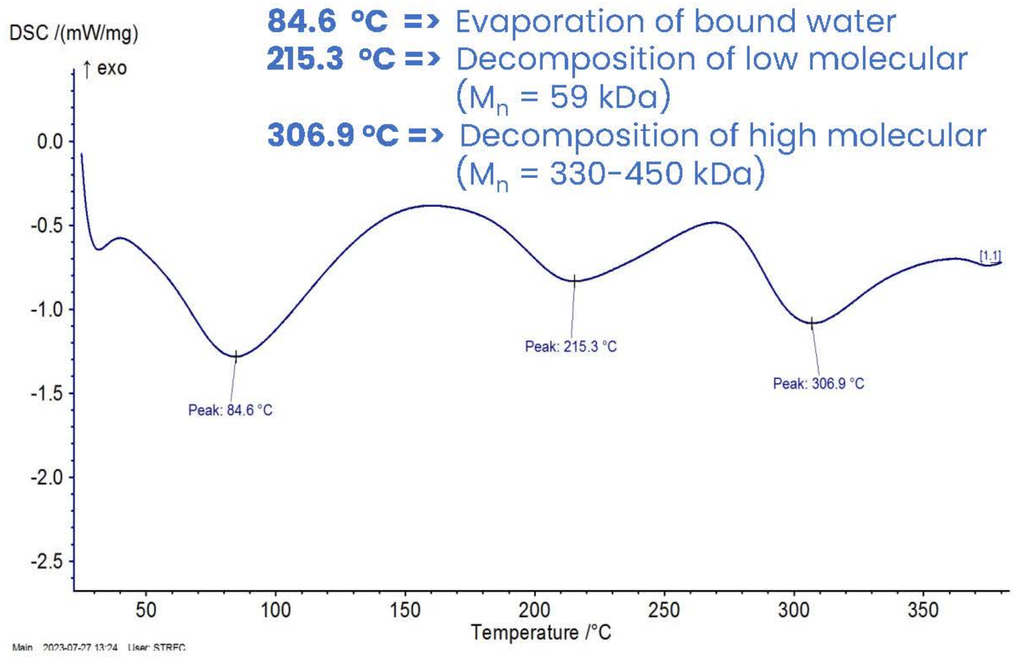


Fig.2. DSC thermograms of the Sericin extract.

Conclusion

Our study demonstrates that the Sericin from silk threads of TT had high antioxidative and the potential to be employed as multifunctional additives in cosmetics, and other fields.

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Reference

[1] A. Intiquilla, K. Jiménez-Aliaga, F. Guzmán, C.A. Alvarez, A.I. Zavaleta, V. Izaguirre, B. Hernández-Ledesma Novel antioxidant peptides obtained by alcalase hydrolysis of Erythrina edulis (pajuro) protein J. Sci. Food Agric., 99 (5) (2019), pp. 2420-2427.

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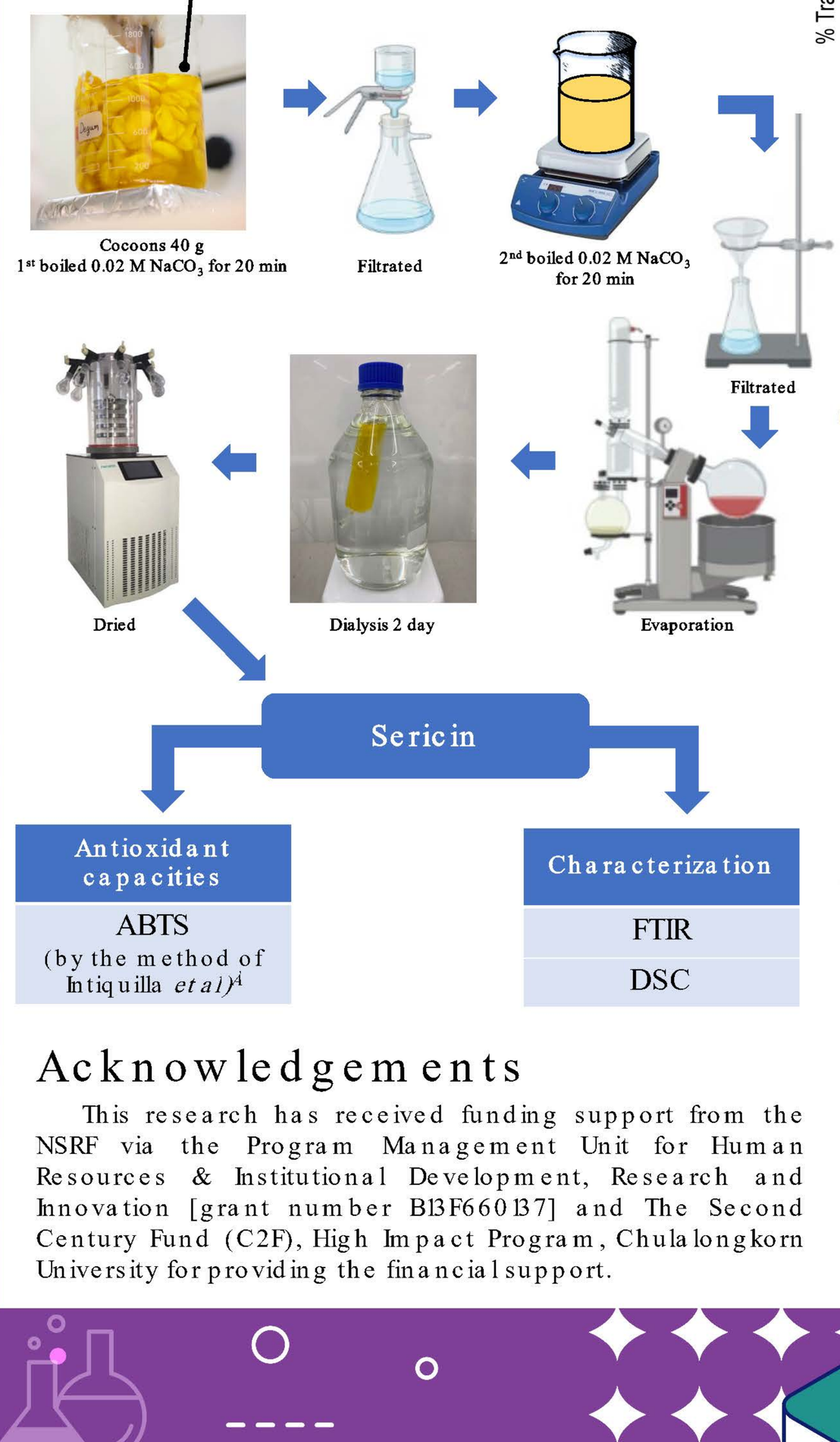
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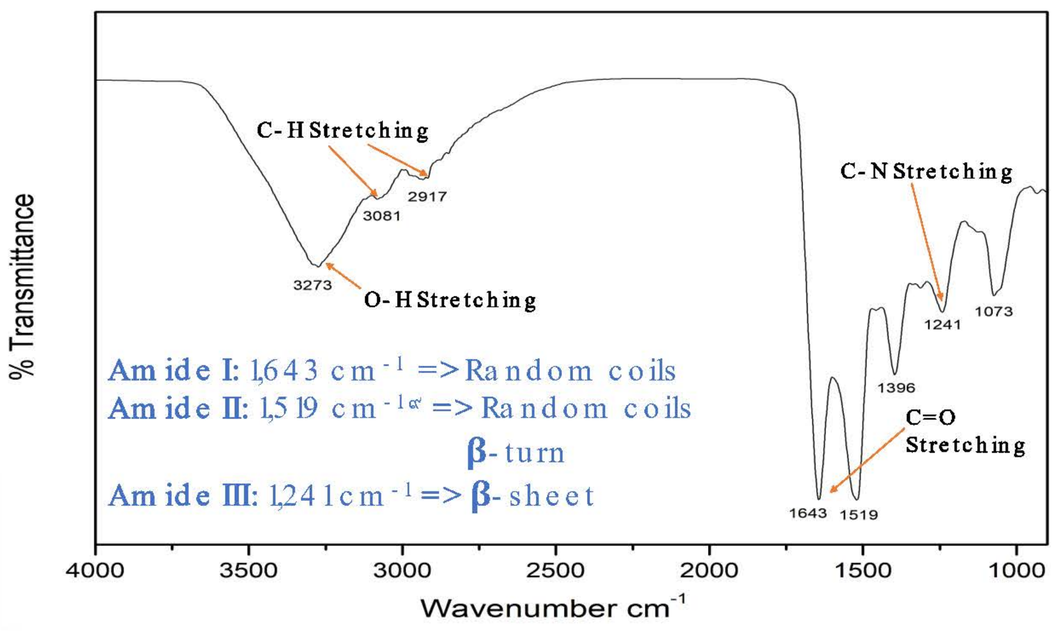


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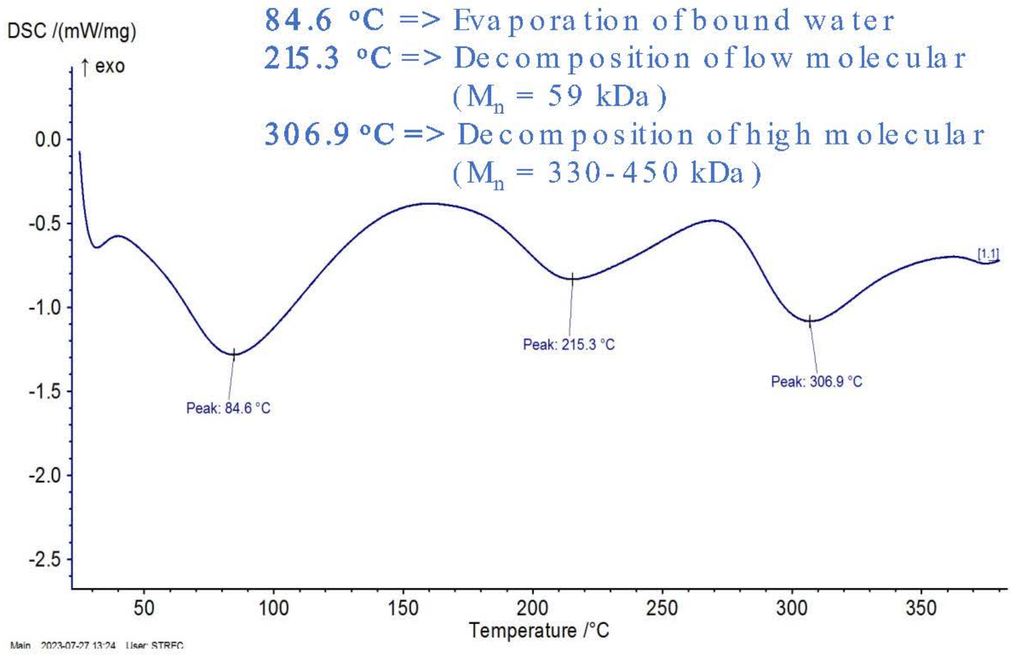


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สู่อุตสาหกรรมแห่งอนาคต

Development and Assessment of an Innovative Plant-Based Vaccine Delivery System Demonstrating Prolonged Immune-Stimulating Properties

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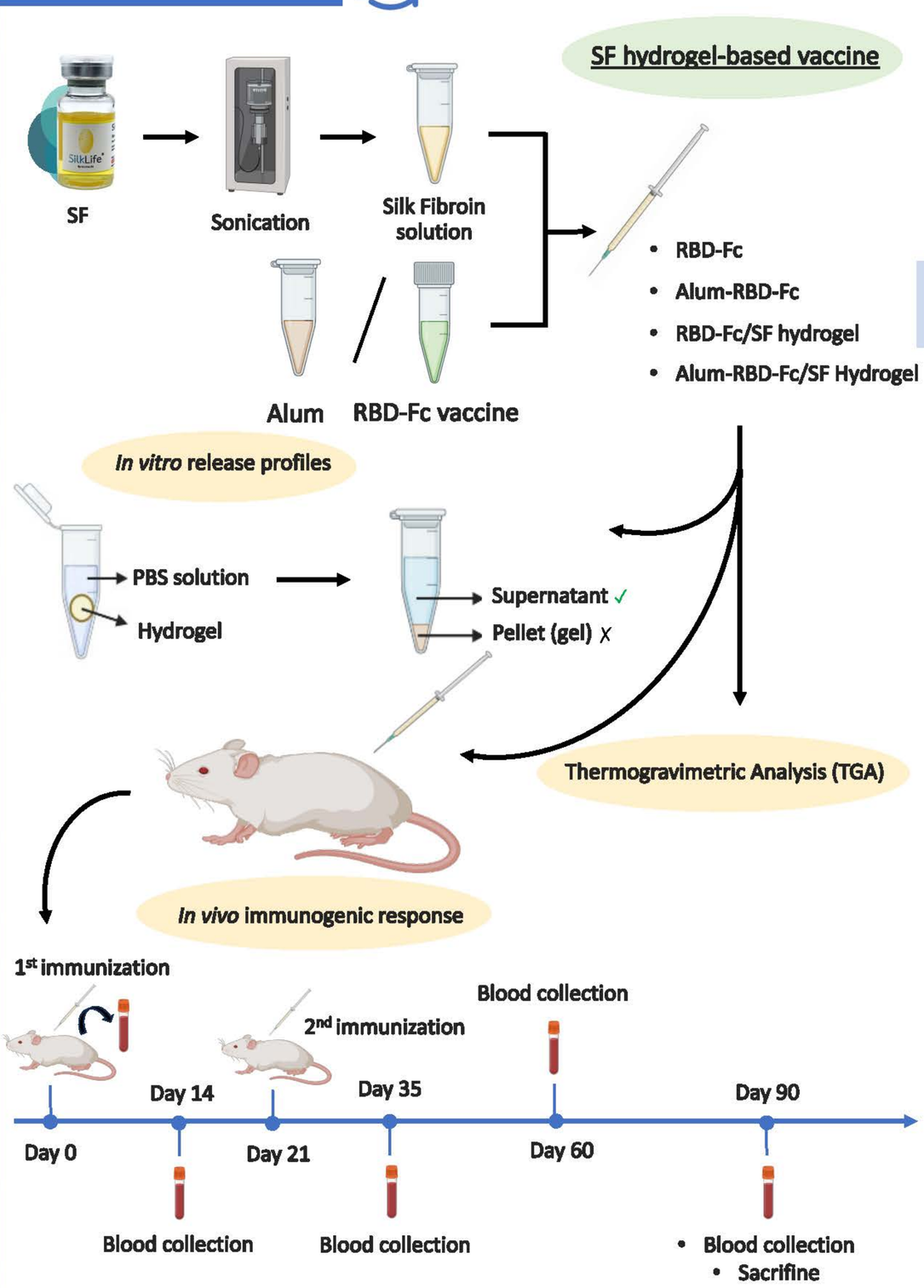
Introduction



Fig. 1. Schematic representation of SF hydrogel-based vaccine formulation.

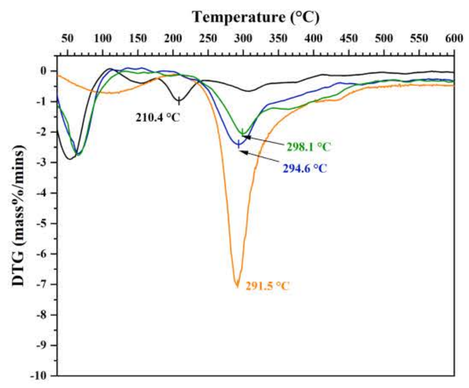
- **A SARS-CoV-2 vaccine, utilizing a subunit protein and proteins to bind receptors on the cell surface, employs RBD-Fc.**
- **The RBD-Fc vaccine requires controlled-release carriers, offering a potential solution for prolonged immunization.**
- **Silk fibroin (SF) is a natural biopolymer isolated from silkworm cocoons. It possesses biocompatibility, biodegradability, and a controllable degradation rate. It can effectively encapsulate drugs, cells, or active molecules.**

Methods



Results & Discussion

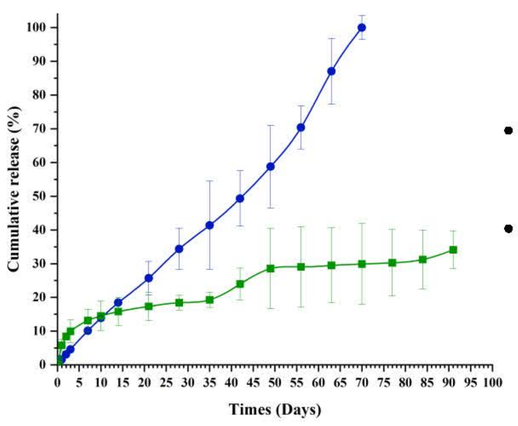
01 Thermal stability of SF hydrogel-based vaccine formulations



- **The higher degradation temperature of RBD-Fc/SF hydrogel demonstrates the protective effect of SF hydrogel against the deterioration of the RBD-Fc vaccine.**

Fig. 2. DTG curves of the RBD-Fc (—), SF hydrogel (—), RBD-Fc/SF hydrogel (—), and Alum- RBD-Fc/SF hydrogel (—).

02 *In vitro* release profiles of SF hydrogel-based vaccine formulations



BSA was utilized as a protein model.

- **Alum-BSA/SF hydrogel, a lower amount of BSA was released.**
- **BSA/SF hydrogel, BSA was released more rapidly.**

Fig. 3. *In vitro* release profiles of BSA/SF hydrogel (●) and Alum-BSA/SF hydrogel (■). Values represented as mean ± SD, n = 3.

03 *In vivo* immunogenic response on SF hydrogel-based vaccine formulations

IgG: stimulate Immunoglobulin immunity
nAbs: neutralize antibodies

- **SF hydrogel-based vaccine formulations demonstrated a long-lasting immune response on Day 60**

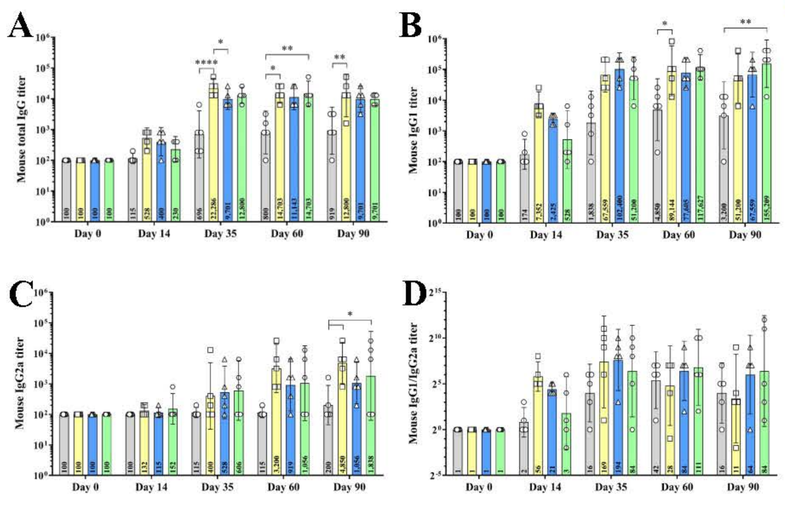


Fig. 4. Titer of SARS-CoV-2 RBD-specific total mouse (A) IgG, (B) IgG1, (C) IgG2a, and (D) ratio of SARS-CoV-2 RBD-specific mouse IgG1/IgG2a titer in sera of mice injected with RBD-Fc (■), Alum-RBD-Fc (■), RBD-Fc/SF hydrogel (■), and Alum-RBD-Fc/SF hydrogel (■) for different time intervals.

Conclusion

- **The SF hydrogel-based vaccine formulations;**
  - ✓ **SF hydrogel against the deterioration of the RBD-Fc vaccine.**
  - ✓ **Controlled and prolonged release of the RBD-Fc vaccine**
  - ✓ **Maintaining immune response concentrations.**
- **SF hydrogel-based vaccine formulation, prolonged and stable immunization can be achieved, potentially eliminating the need for booster immunizations.**

Acknowledgements

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Stability Evaluation of Plant-Produced Angiotensin Converting Enzyme 2 (ACE2)

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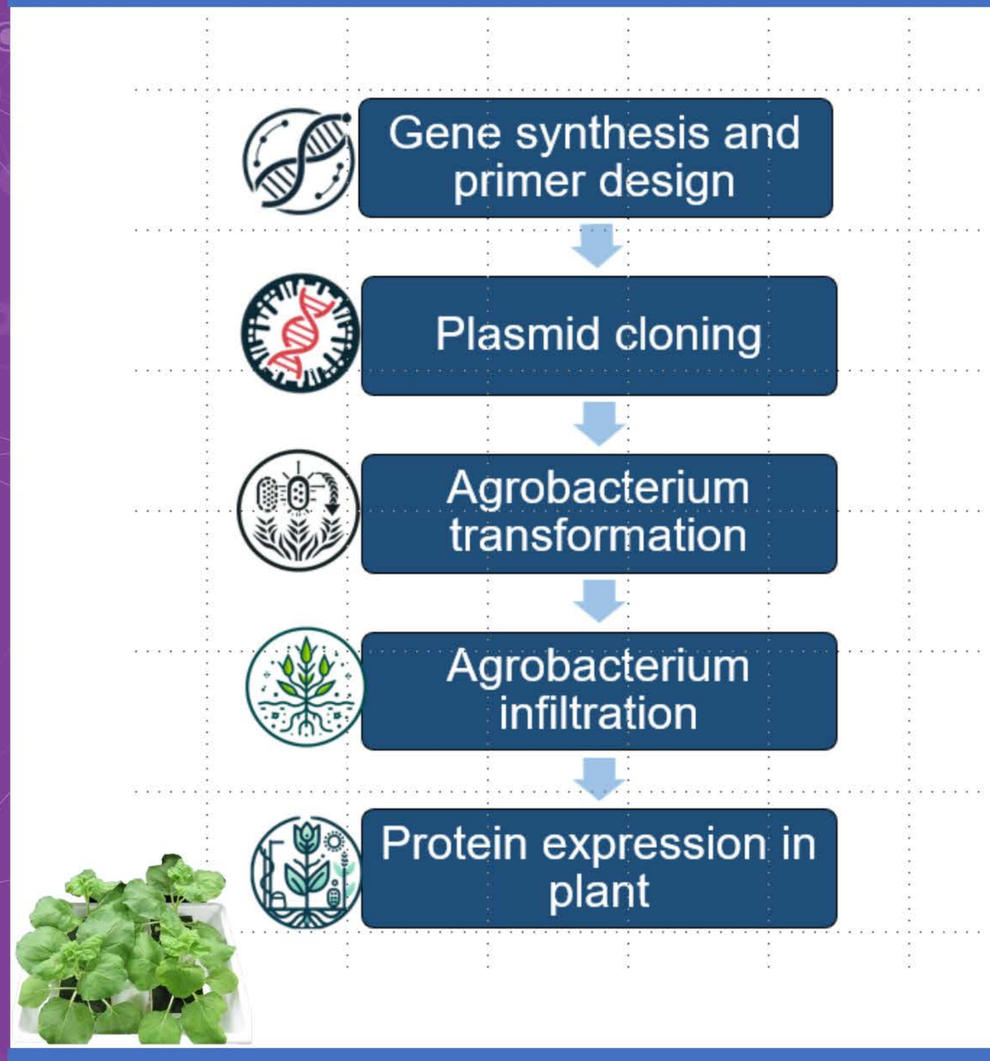
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INTRODUCTION

ACE2 is a promising protein that mimics the structure of angiotensin-converting enzyme 2 (ACE2), exploited by the SARS-CoV-2 virus to invade human cells. ACE2 is being developed as a potential treatment or prophylaxis against SARS-CoV-2 infection, and it shows promise for developing a broad-spectrum antiviral therapy. In this context, evaluating the stability of plant-produced ACE2 is crucial to ensure safety, quality, and effectiveness as a therapeutic agents. By evaluating the stability of the protein, researchers can collect more data on its potential as a drug substance or formulated drug product. This is an important step towards developing effective treatments for viral infections.

Research into product development related to virus infection is essential for protecting public health, strengthening global health security, and protecting vulnerable populations.

EXPERIMENT PROCEDURES

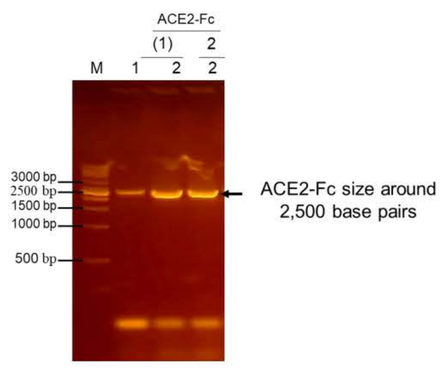


OBJECTIVE

Stability testing evaluates the effect of environmental factors on the product safety, quality, and efficacy of a drug substance or a formulated drug product. Stability testing determines a product's shelf-life and optimal storage conditions.

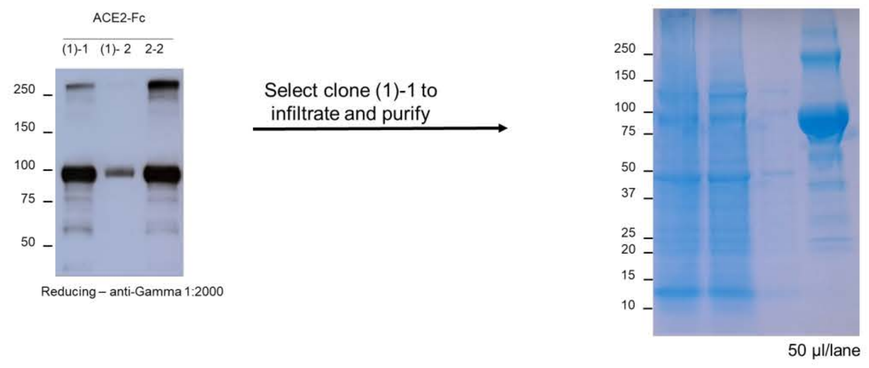
RESULT & DISCUSSION

Colony PCR in Agrobacterium tumefaciens GV3101



PCR detection of ACE2 demonstrated the expression of the ACE2 gene in Agrobacterium in the selective primer.

Protein Expression



Nicotiana benthamiana protein expression was successfully validated, demonstrating the successful production of the protein of interest in this plant platform.

CONCLUSION

The ACE2 has been successfully produced according to plan, with the protein expression demonstrated in Agrobacterium and Nicotiana benthamiana. The protein will be formulated, and its product stability will be evaluated. This next step will ensure that the product meets the standards of quality and effectiveness.

ACKNOWLEDGEMENT

This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137]

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New insight of a Amaryllidaceae plant targets IL-17, the key inflammatory cytokine-induced Benign Prostatic Hyperplasia (BPH)

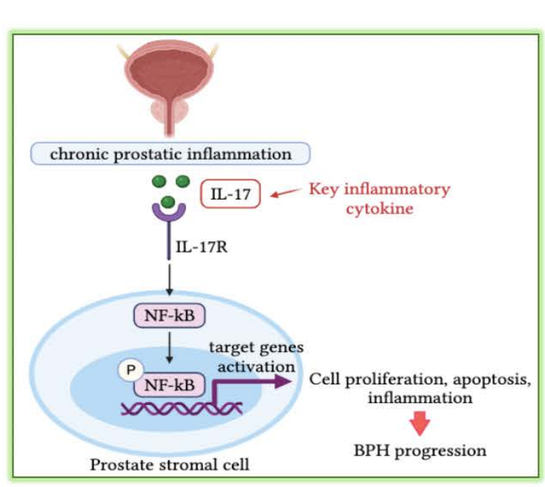
Tamonwan Uttarawichien[1,2], Pithi Chanvorachote[3,4], Wisuwat Thongphichai[1,2], Teerawat Boonsom[1,2], Witchuda Payuhakrit[5], Pasarapa Towiwat[3,6], and Suchada Sukrong[1,2*]

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Introduction

Benign prostatic hyperplasia (BPH), is one of the most common conditions among men aged over 40. Even though BPH is a non-serious condition, patients nonetheless experience urinary tract complications including erectile dysfunction, urinary tract obstruction, retention, infection, and incontinence (1). A major contributing factor to the advancement of BPH is chronic inflammation. Th17 inflammatory cells release IL-17, a crucial inflammatory cytokine that stimulates prostate stromal cell proliferation by activating the NF-kB signal. Prostate stromal cells then secrete IL-8 to further stimulate their own proliferation (2). Existence, a number of medications are available to treat BPH; however, these medications have increased side effects, including decreased libido, gynecomastia, and ϵjaculatory problems (3). As a result, the majority of patients would rather utilize herbal supplements as an alternate kind of medication that has minimal side effects or none at all to treat their BPH symptoms. A few studies reported the effect of Amaryllidaceae plants through inhibition of prostate cell proliferation, which had lycorine, 6α-hydroxybuphanidrine, and crinamidine as bioactive alkaloid constituents (4-5). Nonetheless, the anti-BPH effect of the plants through regulation of IL-17, inflammatory cytokine directly induced prostate stromal cell proliferation has not been discovered yet. Therefore, the purpose of this study is to examine whether the Amaryllidaceae plant can reduce inflammation, which in turn can slow the development of BPH.



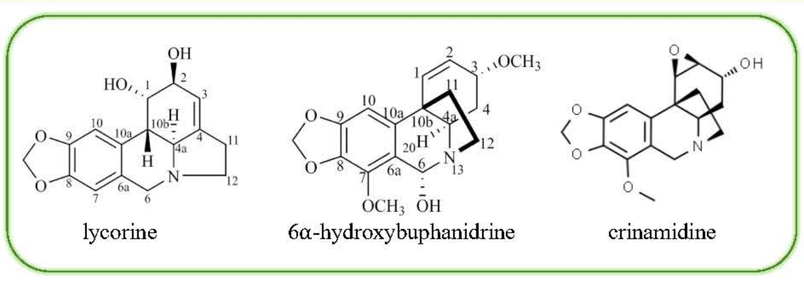
Methods & Results

History used Amaryllidaceae plants for anti-BPH



Amaryllidaceae plants

Previous studies reported anti-proliferation activity of alkaloid compounds extracted from Amaryllidaceae plants against BPH. However, the mechanism underlying their effect has not been clarified yet.



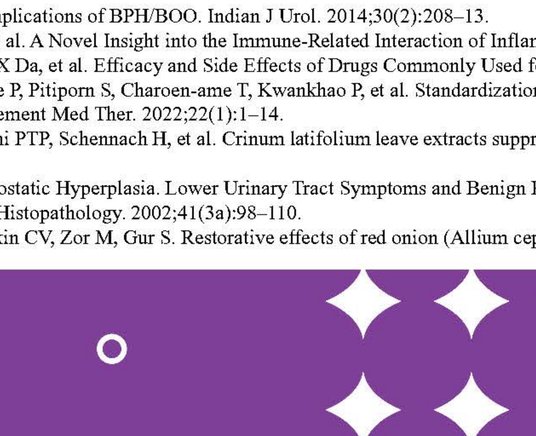
Network pharmacology was used to investigate the potential mechanisms in which these compounds target BPH.

Network pharmacology results demonstrated these compounds from Amaryllidaceae plants have the possibility to inhibit BPH through regulating the IL-17, inflammatory signaling pathway.

(A)

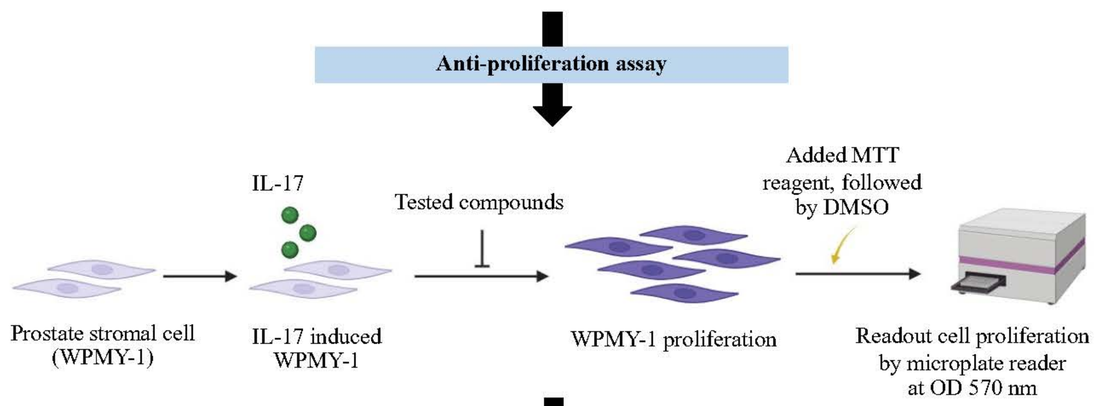


(B)



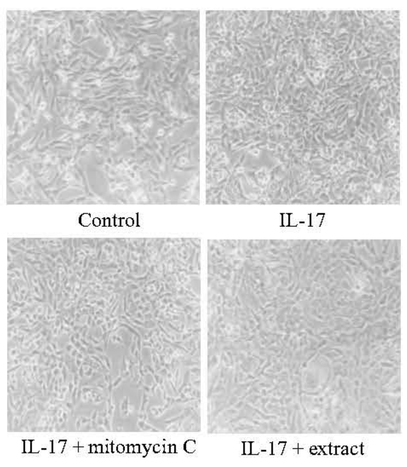
Explore new effect of the Amaryllidaceae plant through IL-17-induced prostate cell proliferation

Anti-proliferation assay was performed to assess the inhibitory effect of the extract through IL-17, a new possibility target of Amaryllidaceae plant for anti-BPH.

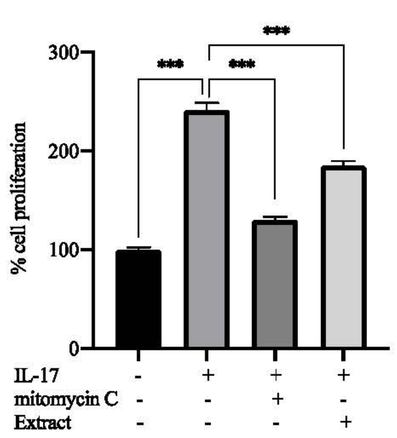


The inhibitory effects of the Amaryllidaceae plant extract on IL-17-induced WPMY-1 proliferation, displaying the population of WPMY-1 cells under an inverted microscope (A). The population of WPMY-1 cells was increased to 241.50±7.05% (p<0.001) when the cells were treated with IL-17 compared to the control group. Whereas the proliferated cells were significantly decreased to 130.22±3.23% (p<0.001) and 184.80±5.16% (p<0.001) when the cells were treated with mitomycin C (positive control) and the extract, respectively (B).

(A)



(B)



Discussion and Conclusion

Many studies have shown Amaryllidaceae plant extracts treating BPH (6–8), but some have found the bioactive components of these extracts act on anti-BPH. Bioactive compounds including lycorine, 6α-hydroxybuphanidrine, and crinamidine have been shown to inhibit prostate cell proliferation (4-5). However, their potential to reduce inflammation related with BPH has not yet been explored. Based on network pharmacology results, we investigated the potential effects of these compounds on the molecular pathways associated with BPH disease. The IL-17 signaling pathway was found to be the top 10 pathways analysis, indicating that these compounds may be able to inhibit BPH by acting on the IL-17 signaling pathway, which is in line with the purpose of this study. Therefore, to study the extract responding in this pathway, anti-proliferation was investigated by using IL-17, a key inflammatory cytokine of the pathway as an stimulator for prostate stromal cell proliferation. Results showed the extract significantly inhibited IL-17-induced prostate stromal cell proliferation. However, effects of the extract on anti-inflammatory contributed BPH will be further studied.

Acknowledgement

We are grateful for laboratory instruments support from the central laboratory at the Faculty of Pharmaceutical Sciences, Chulalongkorn University and the Department of Pathobiology, Mahidol University. This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137] for researcher finance. We also acknowledge the financial support for this research provided by Chulalongkorn University's Fundamental Fund and Herb Guardian Co. Ltd.

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Antiproliferative activity of Zingiberaceae on stromal cells (WPMY-1)

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Introduction

Benign Prostate Hyperplasia (BPH) is one of the most common diseases among middle-aged men. The prevalence of BPH increases with aging. It occurs in 15% to 60% of men aged 40 and older, and the prevalence increases to 70-80% after the age of 80. The most popular form of treatment for BPH is medication, which includes tadalafil, finasteride, and alfuzosin. Unfortunately, pharmacological therapy has a number of side effects, including erectile dysfunction, bleeding, retrograde ejaculation, and urinary tract infections.

As an alternate approach to treating BPH, the use of herbal medication with minimal to no side effects has grown in popularity. The ethnopharmacology of Thailand is predicated on Zingiberaceae for observe the potential anti-BPH.

Methods

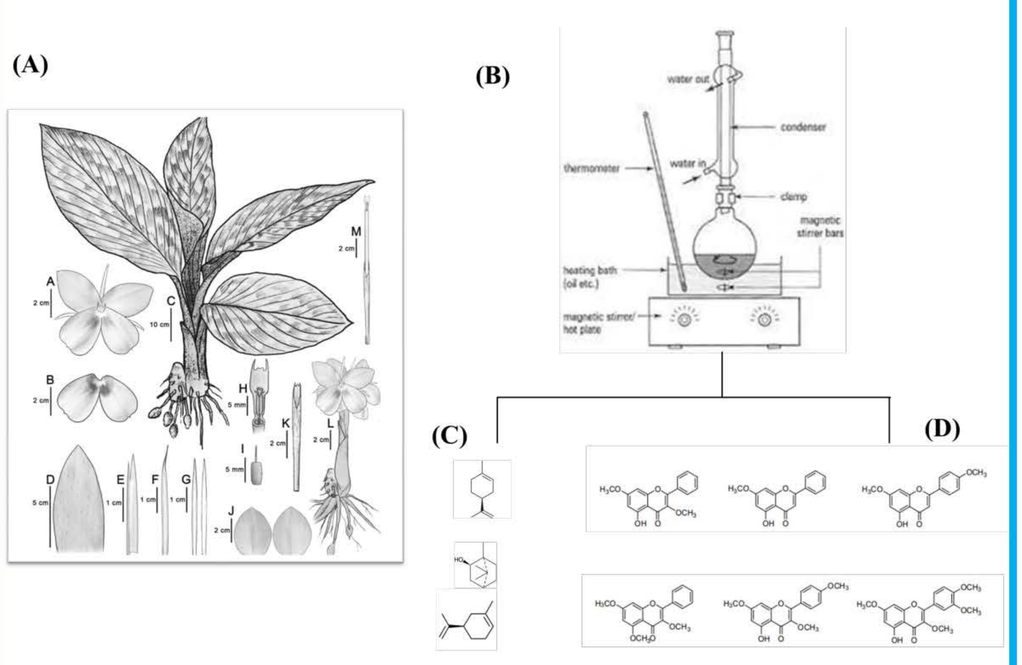


Figure 1. Plant materials and extraction process **(A)** Zingiberaceae **(B)** Reflux extraction **(C)** Terpenoids **(D)** Flavonoids

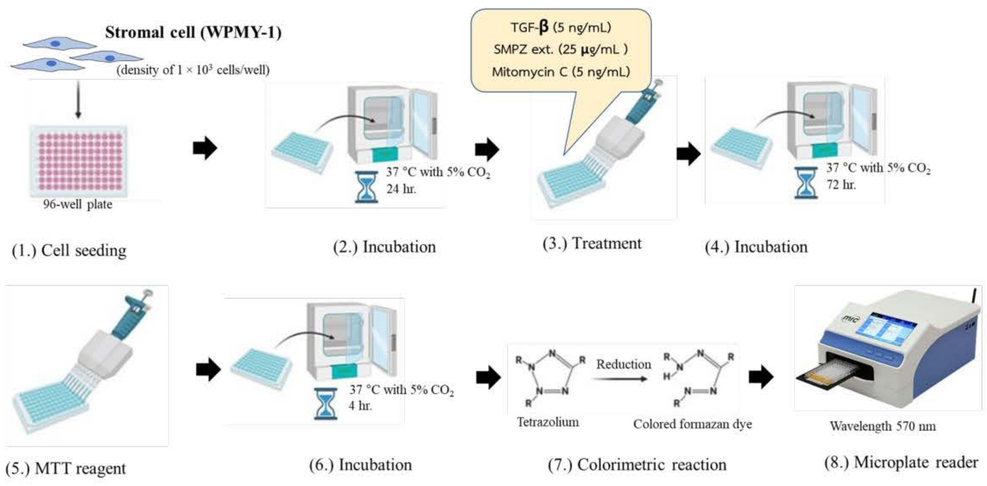


Figure 2. Cytotoxicity and antiproliferative tests

Results and Discussion

The cytotoxicity assay revealed that the maximal nontoxic concentrations of mitomycin C, Zingiberaceae extract were 5 ng/mL, and 25 g/mL, respectively. Hence, the antiproliferative assay was conducted using these doses. The optimal TGF-β concentration employed to promote WPMY-1 proliferation was 5 ng/mL. The findings demonstrated that TGF-β treatment alone greatly boosted WPMY-1 cells' proliferative rate compared to 100% for untreated cells. However, the number proliferated WPMY-1 cells were dramatically decreased after treatment with Zingiberaceae extract. In traditional Thai medicine, Zingiberaceae has been used for the treatment of symptoms related to BPH such as diuretic. A recent study revealed that in BPH, the proliferation of stromal cells is four times greater than that of epithelial cells. Because the proliferation of stromal cells plays an important role in BPH, prostate stromal cells (WPMY-1 cells), which are a predominant cell type involved in BPH progression, were used in this study. A previous study reported the effects of TGF-β on the proliferation of BPH stromal cells. At low doses, TGF-β promotes prostate stromal cell proliferation, while high doses of TGF-β potently induce cell growth arrest. Therefore, TGF-β was used as an inducer of WPMY-1 proliferation. Our results demonstrated that TGF-β at a concentration of 5 ng/mL significantly promoted WPMY-1 proliferation. Interestingly, TGF-β-induced WPMY-1 cell proliferation was reduced by treatment with Zingiberaceae extract (Fig. 4). To create reproducible results and investigate the chemical composition of this extract, which may be related to its antiproliferative activity, standardization of the Zingiberaceae extract was further carried out.

Results

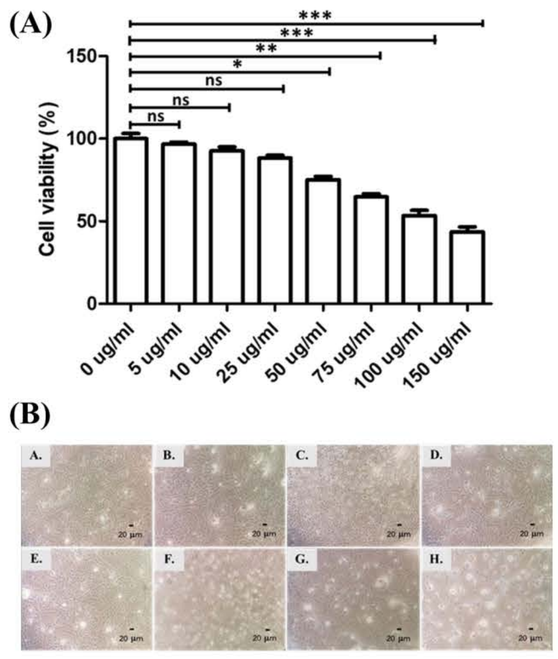


Figure 3. Determination of the maximum nontoxic dose of the tested compounds, WPMY-1 cell viability was investigated by the MTT method. **(A)** Cell viability and bar graph revealing the percent cell viability represented as the means ± SD (ns = non-statistically significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). **(B)** Images of WPMY-1 cells treated with different Zingiberaceae extract. All images were captured at 100 × magnification; scale bar = 20 μm.

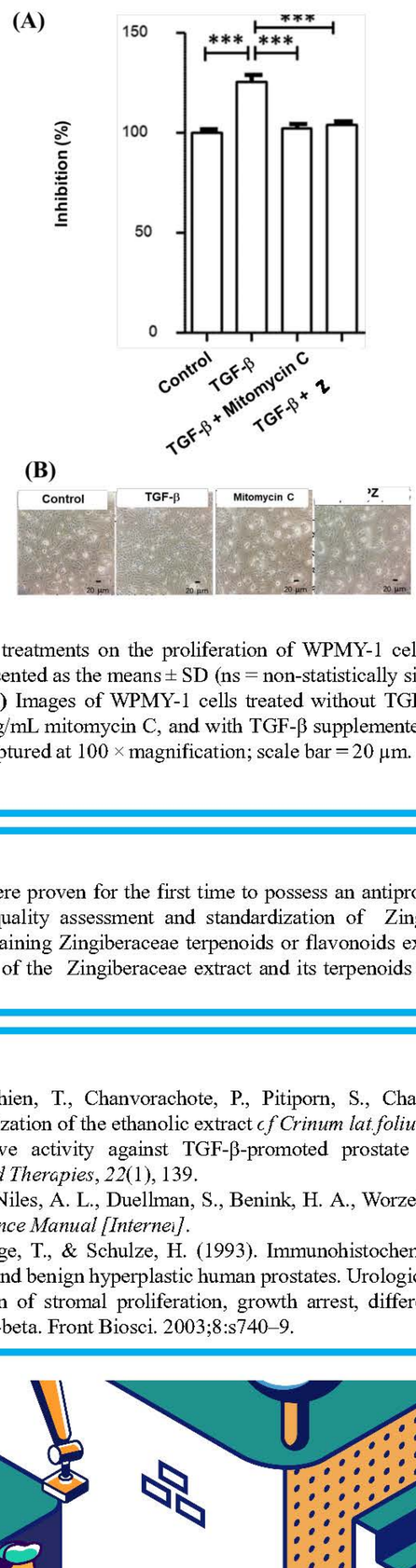


Figure 4. Effects of different treatments on the proliferation of WPMY-1 cells. **(A)** Bar graph revealing the percent cell proliferation represented as the means ± SD (ns = non-statistically significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). **(B)** Images of WPMY-1 cells treated without TGF-β (control), with TGF-β, with TGF-β supplemented with 5 ng/mL mitomycin C, and with TGF-β supplemented with 25 μg/mL Zingiberaceae (Z) extract. All images were captured at 100 × magnification; scale bar = 20 μm.

Conclusion

Zingiberaceae extract were proven for the first time to possess an antiproliferative effect on TGF-β-treated WPMY-1 cells. However, The quality assessment and standardization of Zingiberaceae extract raw materials, extracts, and herbal products containing Zingiberaceae terpenoids or flavonoids extract was further carried out. The underlying molecular mechanism of the Zingiberaceae extract and its terpenoids or flavonoid on BPH needs to be elucidated.

Acknowledgements

We are grateful for laboratory instruments support from the central laboratory at the Faculty of Pharmaceutical Sciences, Chulalongkorn University. This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660137].

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