

Hong Kong Student Science Project Competition 2022

Template of Extended Abstract (Investigation)

(Word Limit: 1,000 words, Pages: 2 pages only)

Team Number: SBBC117

Project Title: 破解「修皮」之謎 A study of wound healing using model of cultured fibroblasts

Project Type: Investigation

To our best knowledge and after thorough literature research, as at __31 / __1 / 2022__, there are / are no* similar works. If there are, the reference links are as below:

The enhancement our project has made for the existing related products or research is summarized as below:

*Please delete if not applicable. HKSSPC values the originality of works. Students must conduct literature research thoroughly to ensure that their works are unique, and to list relevant reference materials to complement the research or invention.

I. Background

- Provide background information of project and/or state the problem to tackle
- Provide highlights of the **literature review** with the support of pertinent and reliable references
- Provide an overview of work and mention the **research gap that the project is trying to fill**

According to research data, it is estimated that 1 to 2 % of the population will experience a chronic wound during their lifetime in developed countries. Patients who have chronic disease, their blood circulation is usually poor, and nutrients cannot be sent to the wound to assist in healing. The wound healing mechanism in the human body cannot play an effective role due to lack of nutrition. To help protect these people from wound infection, our study was to explore any potential sources of natural phytochemicals which could speed up wound healing.

Our group would like to launch a wound healing study by exploring the ability of natural phytochemicals to enhance the growth and migration of fibroblast cell over the wound area. The efficacy of the five major ingredients in Huang Long Gao, Amur Corktree Bark (黃柏), Chinese Angelica (當歸), Baikal Skullcap (黃芩), Rhubarb (大黃) and Common Gardenia Fruit (生梔子) in reducing the wound size was investigated time-dependently under different conditions:

- (1) Dose-dependent studies: at varying concentrations of samples
- (2) Exclusion Test: removing one of the components in the combined treatments
- (3) Synergy Test: varying combination of samples

Fibroblasts are critical in supporting normal wound healing, involved in key processes such as breaking down the fibrin clot, creating new extracellular matrix (ECM) and collagen structures to support the other cells associated with effective wound healing, as well as contracting the wound. Therefore, it commonly provides *in vitro* model for studying wound healing effects. Wounds with standard size made by a micropipette tip were treated in this study.

II. Objectives

- State the **aim(s)** of project
- To investigate the dose-dependent effect of Amur Corktree Bark, Chinese Angelica, Baikal Skullcap, Rhubarb, Cape Jasmine at varying concentration of samples
- To investigate any exclusion among the five chinese medicine samples by removing one of the components in the combined treatment
- To investigate any synergistic effect among the five chinese medicine samples by testing varying combination of samples
- To propose a new formula for making a wound healing ointment with natural phytochemicals

III. Hypothesis

- Propose an explanation for a phenomenon and stating how the **hypothesis** can be tested by experiments
1. The five major ingredients in Huang Long Gao, Amur Corktree Bark (黃柏), Chinese Angelica (當歸), Baikal Skullcap (黃芩), Rhubarb (大黃) and Common Gardenia Fruit (生梔子) exert stimulatory effect on the proliferation of Fibroblast.
 2. The stimulatory effect of the samples varies with different concentrations.
 3. The stimulatory effect of the samples varies with different combinations.
 4. Exclusion among the five medicine samples affects the proliferation of Fibroblast.
 5. Wound contraction is the most critical step in the wound healing process.

IV. Methodology

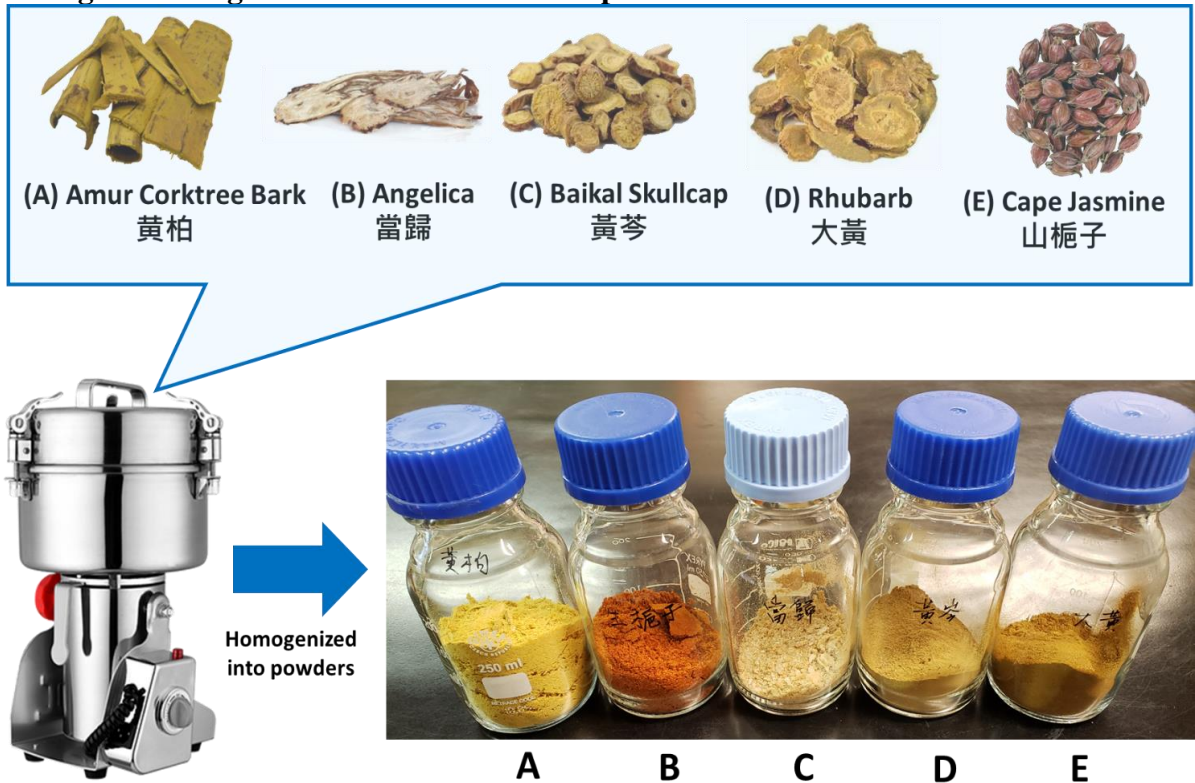
- List out the materials used
- Describe the **experimental protocol** including the set-up of **control experiment** (if any), **repeated experiment** (if any), and its scientific theory
- Indicate with the support of reasons, the **analysis** used in the investigation

Major materials used:

1. The five herbal ingredients, including Amur Corktree Bark (黃柏), Chinese Angelica (當歸), Baikal Skullcap (黃芩), Rhubarb (大黃) and Common Gardenia Fruit (生梔子).
2. Sterilized homogenizer
3. DMSO solution
4. Fibroblast cell
5. DMEM medium
6. 96-well microplate
7. p20 pipette tip

Experimental steps:

1. Extracting Active Ingredients from Herbal Samples



2. Further dilution from the stock solution

1

1 g Sample

extraction in 2ml DMSO overnight

Centrifugation at 1500 rpm for 5 min

Collect supernatant

Stored as 500 mg/ml Sample Stock

2

(2a) 10 mg/ml 5 mg/ml 2.5 mg/ml

(2b) 100 μ l diluted sample stock

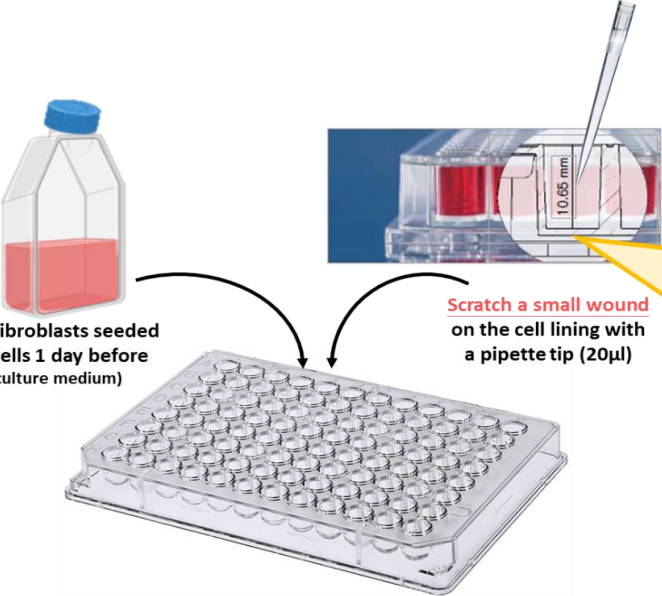
Each well containing 100 μ l fibroblasts seeded into wells 1 day before (in culture medium)

Further dilution:

	Sample stock conc.	10 mg/ml	5 mg/ml	2.5 mg/ml
(2a) 500mg/ml Sample Stock	100 μ l	50 μ l	25 μ l	
Culture medium	5 ml	5 ml	5 ml	
(2b) Final sample conc. in wells	5 mg/ml	2.5 mg/ml	1.25 mg/ml	
DMSO conc. in wells	1%	0.5%	0.25%	

3.

Making wound on the cell lining



100 μ l fibroblasts seeded into wells 1 day before (in culture medium)

Scratch a small wound on the cell lining with a pipette tip (20 μ l)

Length : 65.39 μ m

Studying wound recovery upon sample treatment by

- evaluating **wound size**
- conducting **morphological studies**

Session A


Dose-dependent Treatments

		5 mg/ml					2.5 mg/ml					
	Control	Sample A	Sample B	Sample C	Sample D	Sample E	Sample A	Sample B	Sample C	Sample D	Sample E	
A												
B												
C												
D												
E												
F												
G												
H												

1.25 mg/ml

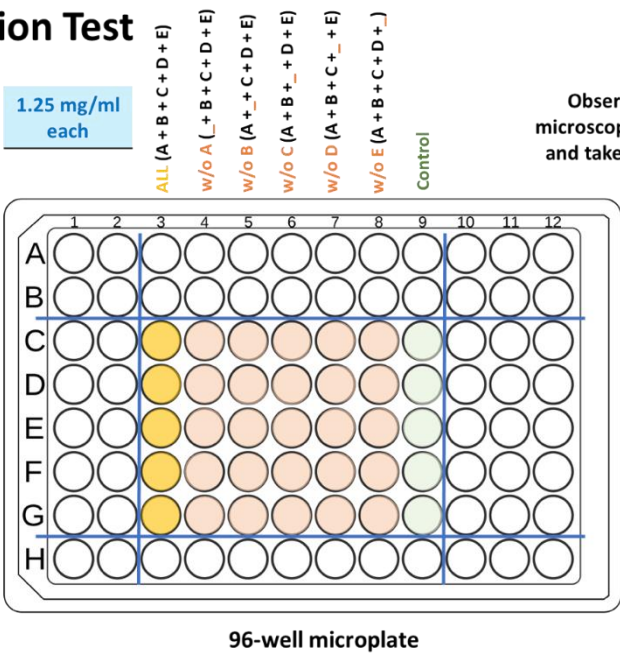
96-well microplate

Observe under the inverted microscope after 12h of incubation and take photos of the cell lining



Session B

Exclusion Test

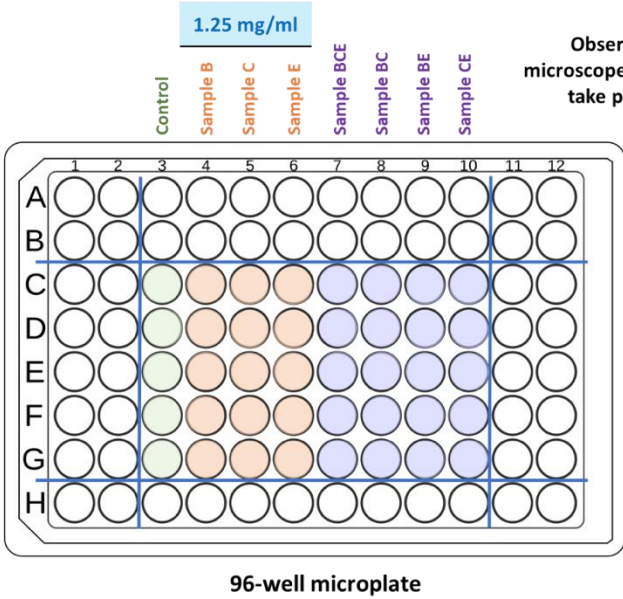


Observe under the inverted microscope after 24h of incubation and take photos of the cell lining



Session C

Test for Synergy



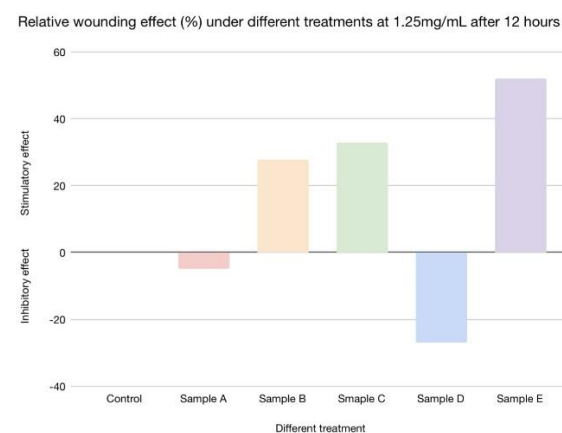
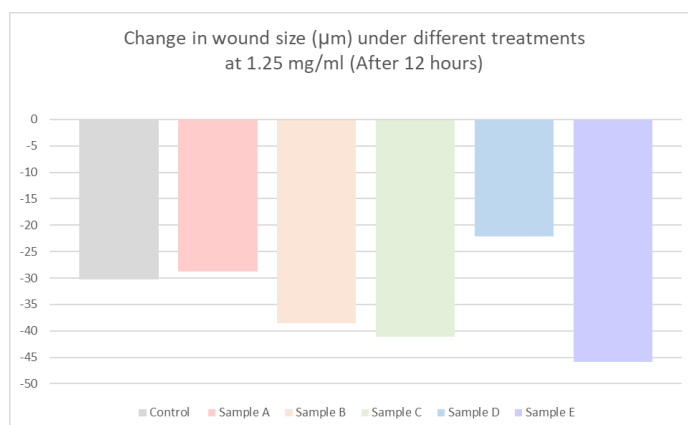
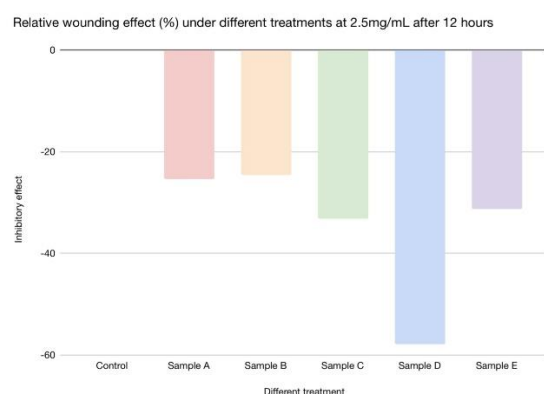
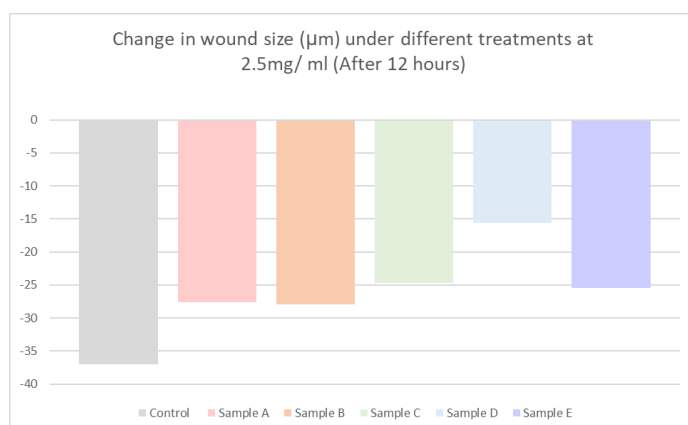
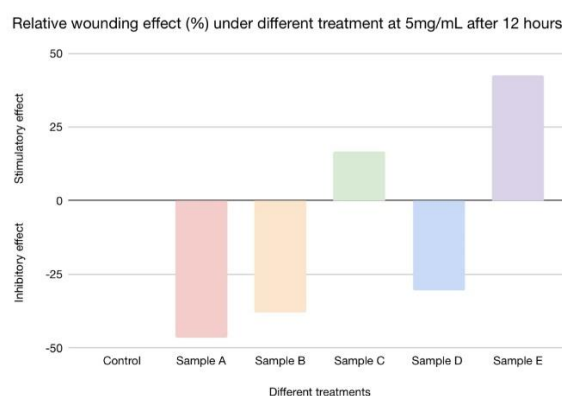
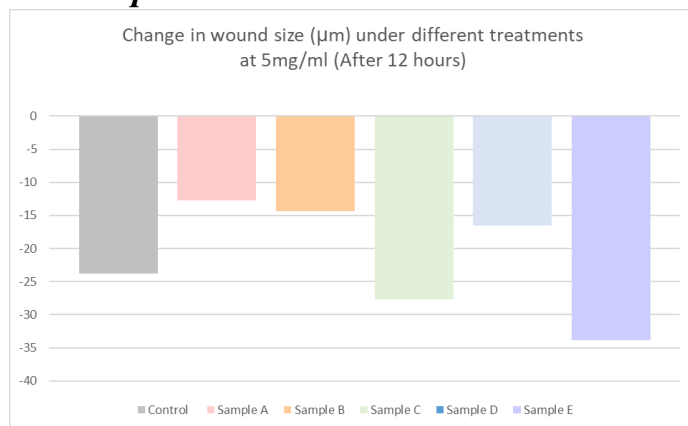
Observe under the inverted microscope after 8h of incubation and take photos of the cell lining



V. Results

- Present the **data** with figures, tables or photos
- **Data analysis** (if any, with emphasis on data reliability and the reproducibility based on statistics)
- Interpret the results and its implication
- Discuss **limitation** and compare with existing related works (if any)
- Discuss the importance or impact of the research and how it is applicable to real problems

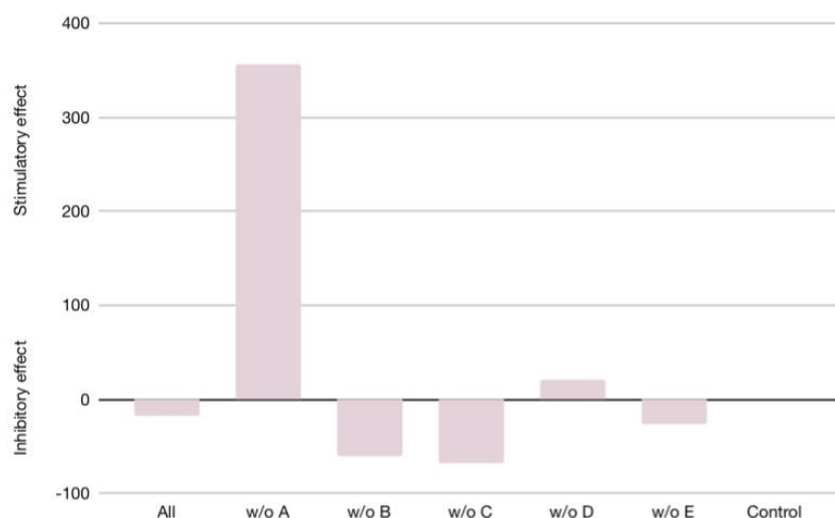
Dose-dependent Studies:



Finding: Concentration of 1.25 mg/ml is the best
Samples B, C and E are selected with their significant healing effect

Exclusion Test:

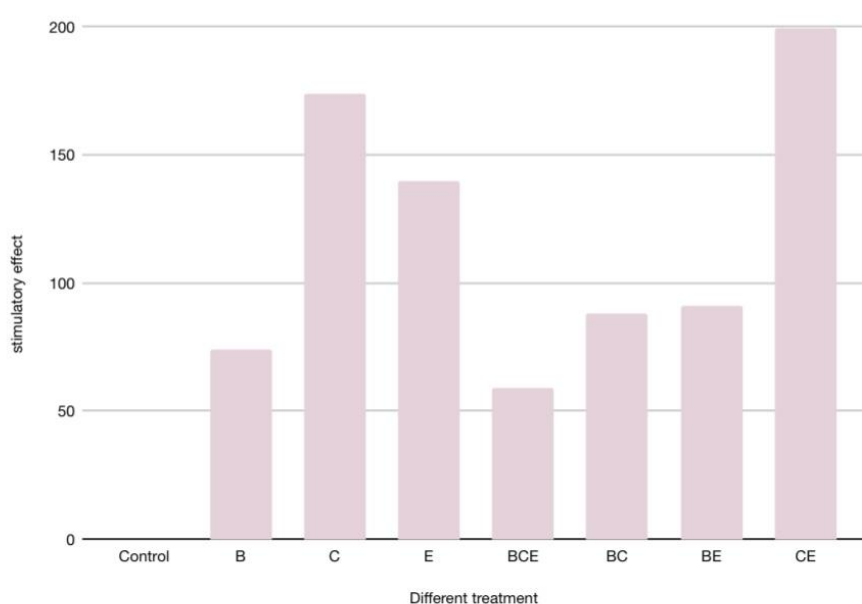
Relative wounding effect under different treatments at 1.25mg/ml after 24 hours



Finding: Samples without A and that without D can give better effect, thus A and D are eliminated

Test for Synergy:

Relative wounding effect (%) under different treatments at 1.25mg/mL after 8 hours



Finding:

- (1) Cells took at least 4 hours to adapt to the treatment with samples
 - (2) No synergistic effect among samples is found
 - (3) Sample B improved the healing effect the best among all samples
- $BCE = 0.33 + 0.33 + 0.33$
(not $1 + 1 + 1$)

VI. Conclusion

- Make a **data-driven** conclusion of the project and the way forward of the research
- Justify if the proposed project meets the objective(s)

It showed that combined group CE had the most efficacious performance in wound healing on human fibroblast cells. Although, significant synergistic effect was not seen in any actions among samples, this project was still rewarding since it was the basis for further development of wound healing ointment with an improved formula of **Baikal Skullcap (黄芩)** and **Common Gardenia Fruit (生梔子)** at 1.25mg/ml in an *in vitro* model of human fibroblast cells.

☐ Our project is developed based on our school's previous project and the enhancement is as below: