

**IECBM  
2022**

# The 2nd International Electronic Conference on Biomolecules: BIOMACROMOLECULES AND THE MODERN WORLD CHALLENGES

01-15 NOVEMBER 2022 | ONLINE

## Pipeline to develop and characterize a potential regenerative topical treatment based on lavender essential oil and CW49 peptide

**GINIB**

Grupo de Investigación en  
Nanobiomateriales, Ingeniería  
Celular y Bioimpresión

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# Healing treatments

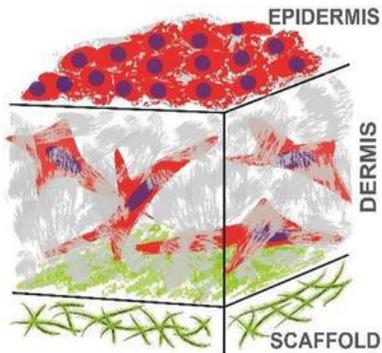
- In the USA chronic wounds affect about 6 million people per year.
- In Colombia, a prevalence of 10% in diabetic and venous disorders patients.



## Current treatments



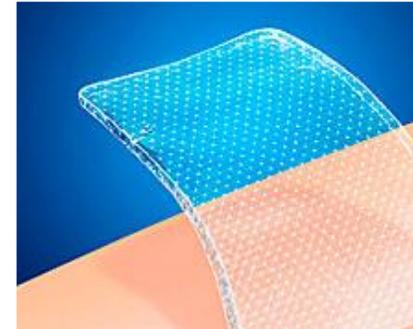
Negative pressure wound therapy



Scaffolds



Biomembranes

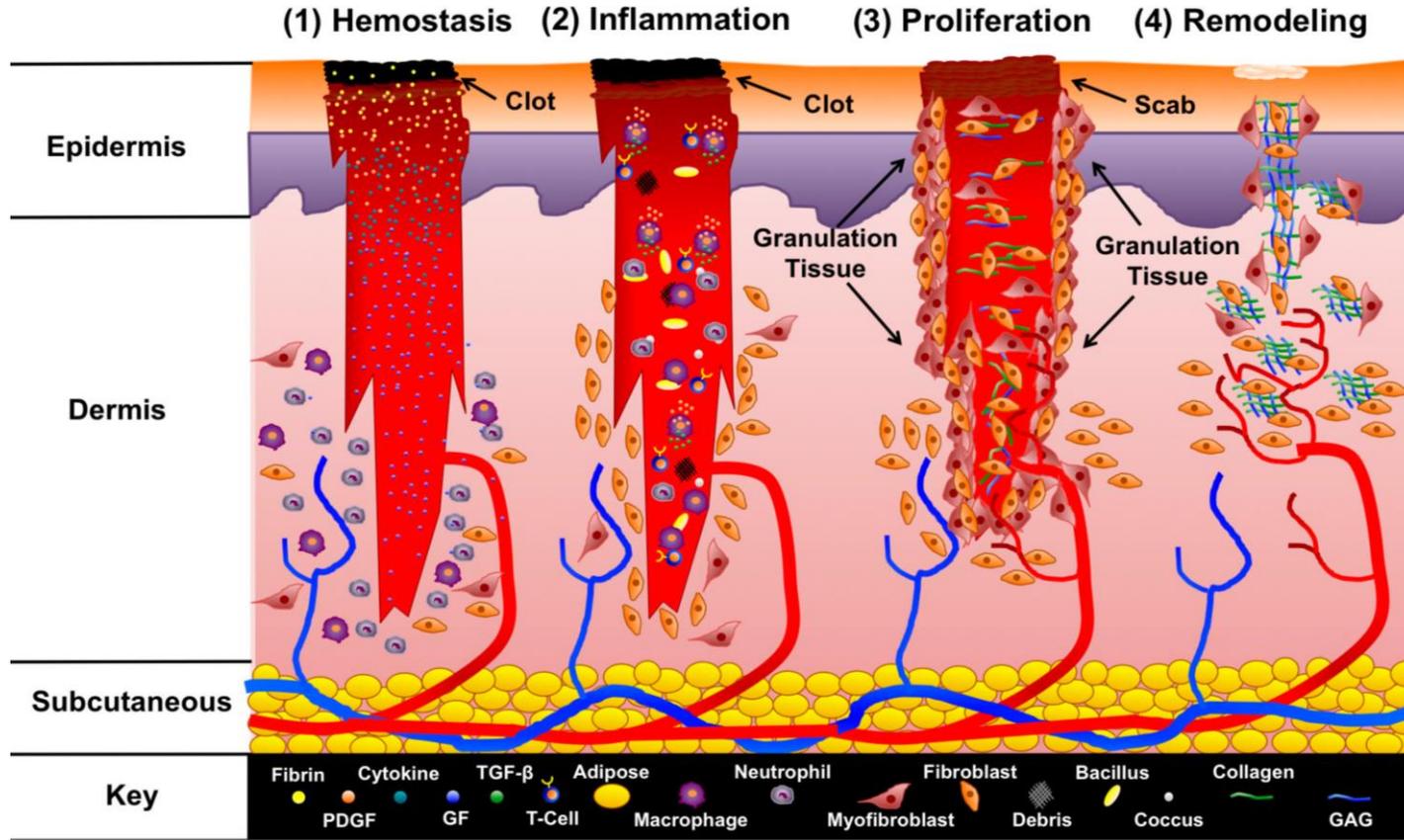


Hydrocolloids dressings



Foams

# Wound healing natural process



If proper healing is not followed, it can cause:



Infections, amputations, cellulitis, abscesses, necrotizing fasciitis, among others.

# Lavender essential oil



*Lavandula angustifolia*

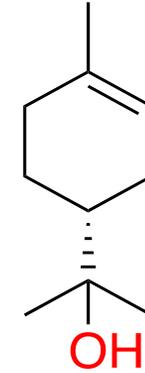
## Properties

### Wound healing improvement

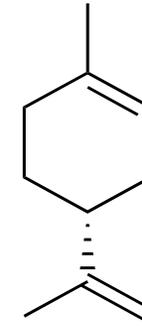
- Accelerate the process of granular tissue formation
- Induction of TGF- $\beta$  expression

- Anti-inflammatory
- Immunomodulatory
- Antinociceptive
- Antimicrobial

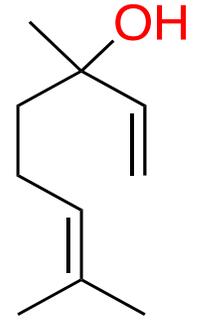
## Compounds



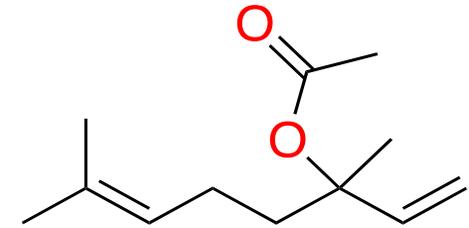
$\alpha$ -terpineol



D-limonene



(-)-linalool



Linalyl acetate

# CW49 peptide

## Properties

### Wound healing improvements

- Accelerate the angiogenic process and the cellular activity

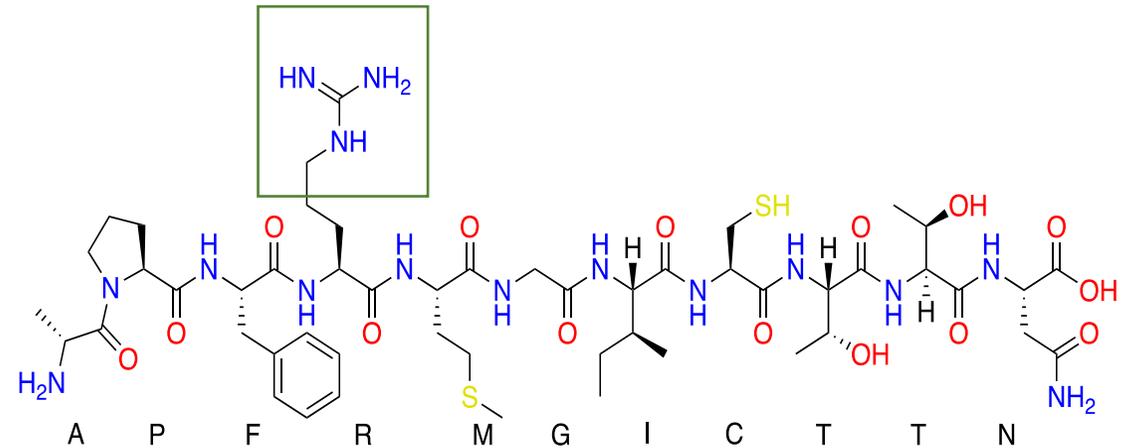
- **Antimicrobial**
- Antiviral
- Anticancer



*Odorrana grahami*

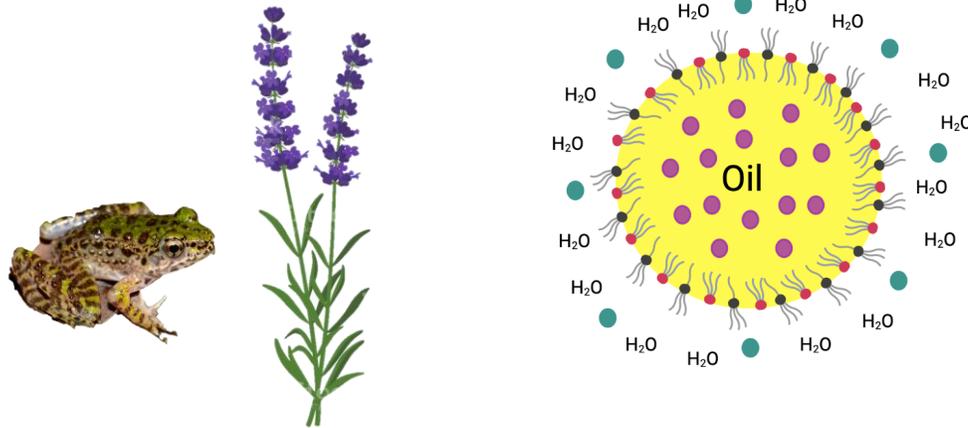
## Structure

- Composed of 11 AA
- molecular mass: 1.2kDa
- Isoelectric point: 8.29
- Charge: +1



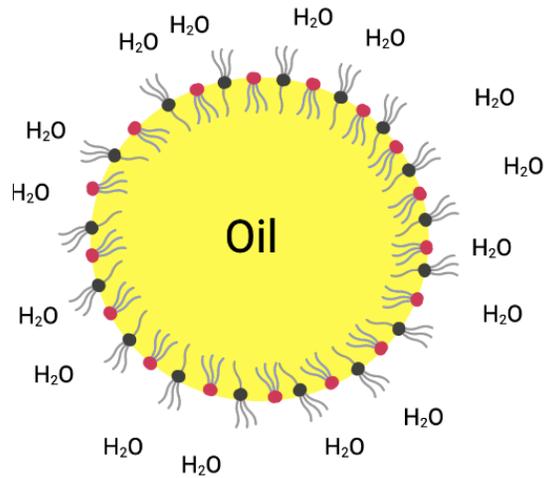
## Main objective

Develop stable emulsions from lavender extract and CW49 peptide for regenerative assays on a 2D human keratinocyte monolayer.



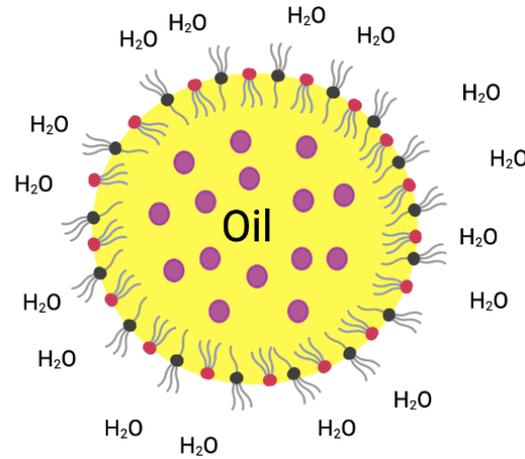
# Methodology O/W emulsions

Objective: Develop stable emulsions from lavender extract and CW49 peptide for regenerative assays on a 2D human keratinocyte monolayer.



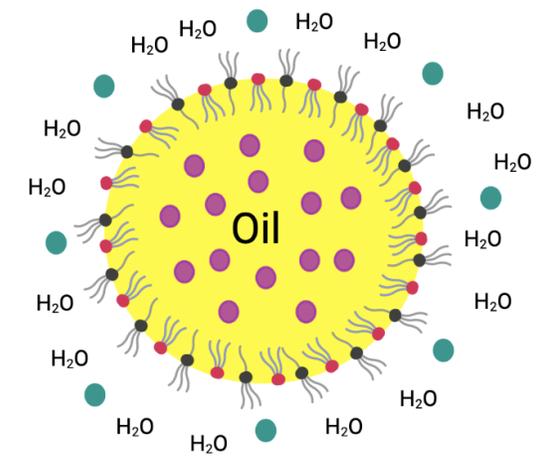
Control emulsion

- Tween 20
- Carbopol
- Span 80



Lavender emulsion

- Lavender oil
- Tween 20
- Carbopol
- Span 80

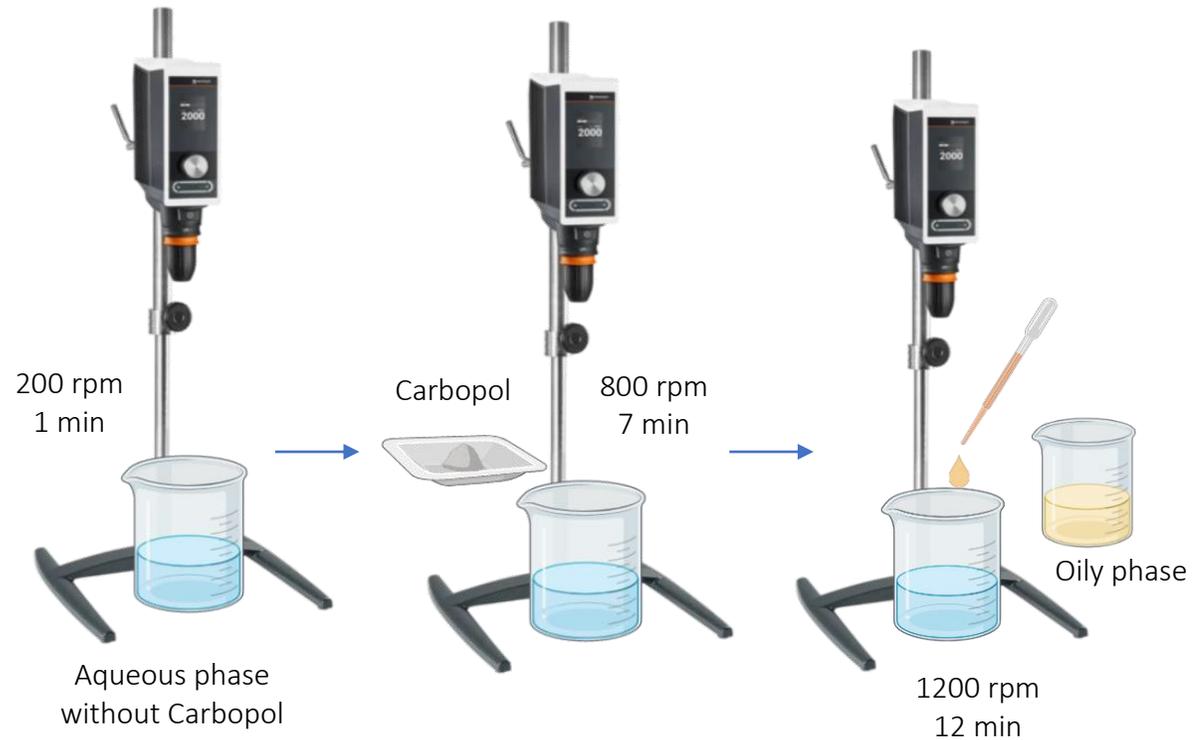


Peptide CW49- Lavender emulsion

- Lavender oil
- CW49 peptide
- Tween 20
- Carbopol
- Span 80

## Methodology O/W emulsions

## Synthesis of the emulsions



Emulsion	Control	Lavender	Lavender-peptide
<b>Composition ( w/w%)</b>			
<i>Oily phase</i>			
Essential oil	-	0.25	0.25
Span 80	1.60	1.60	1.60
Mineral oil	8.40	8.15	8.15
<i>Aqueous phase</i>			
CW49 peptide	-	-	0.2
Tween 20	2.40	2.40	2.40
Carbopol	0.3	0.3	0.3
Triethanolamine	0.6	0.6	0.6
Water	86.70	86.70	86.50



Lavender    Control    Lavender + CW49  
peptide

# Physicochemical characterization

Objective: Determine the physicochemical properties of the CW49 peptide and lavender essential oil emulsion

## Particle size distribution



MasterSizer® 3000

Refractive index:  
Water: 1.33  
Emulsions: 1.47

## Rheological analysis



### Frequency test:

- Storage and loss modulus.
- Frequency sweep with 1% Strain between 0.01-1Hz with 10 points per decade.

### Flow test:

- Viscosity and share stress.
- Frequency between 0.1-100Hz.

## Emulsion stability

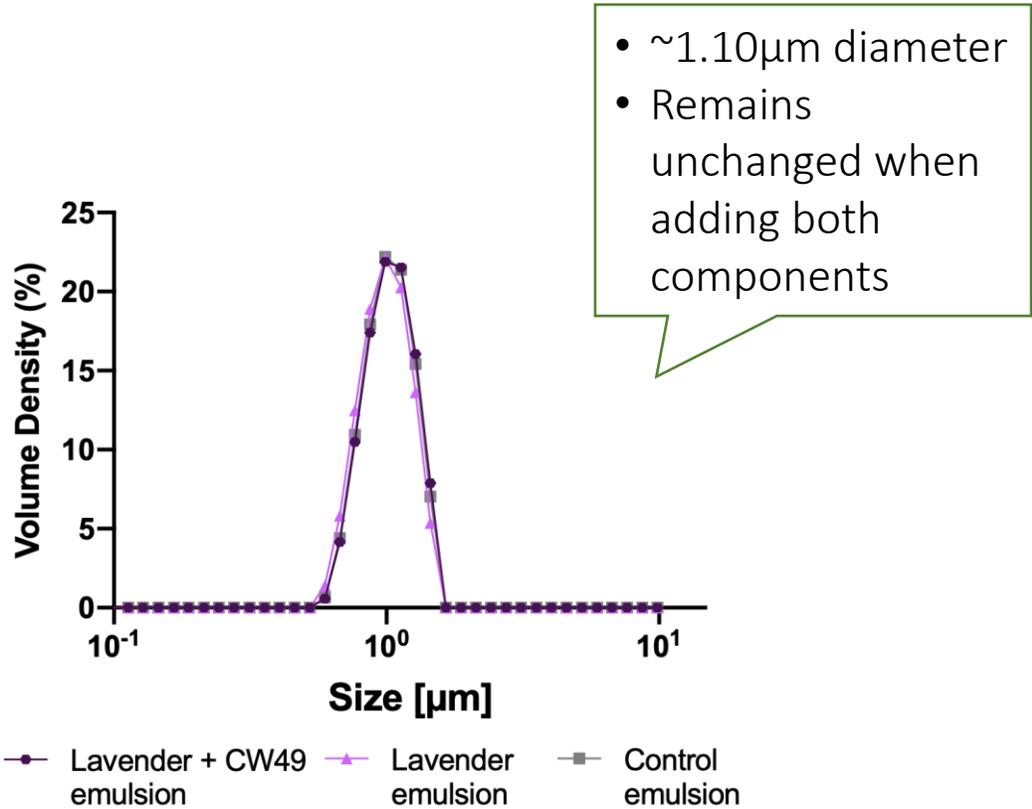


Turbiscan

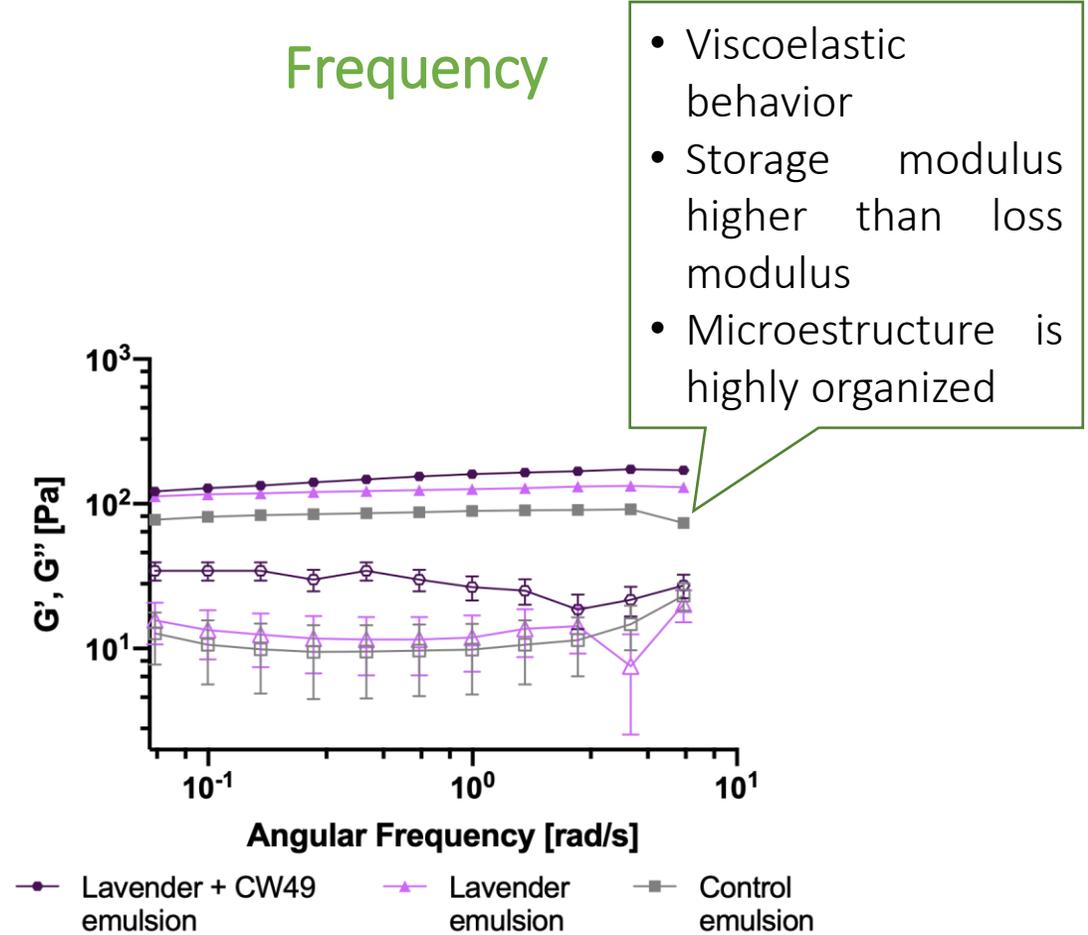
At 25°C 10 sweeps  
per sample

# Physicochemical Characterization

## Particle size distribution

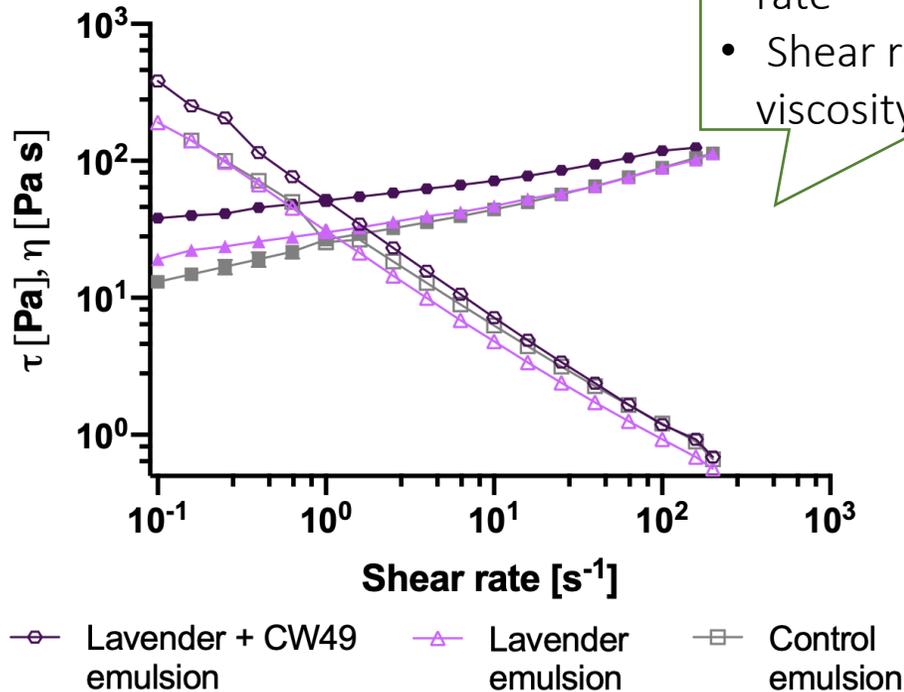


## Frequency



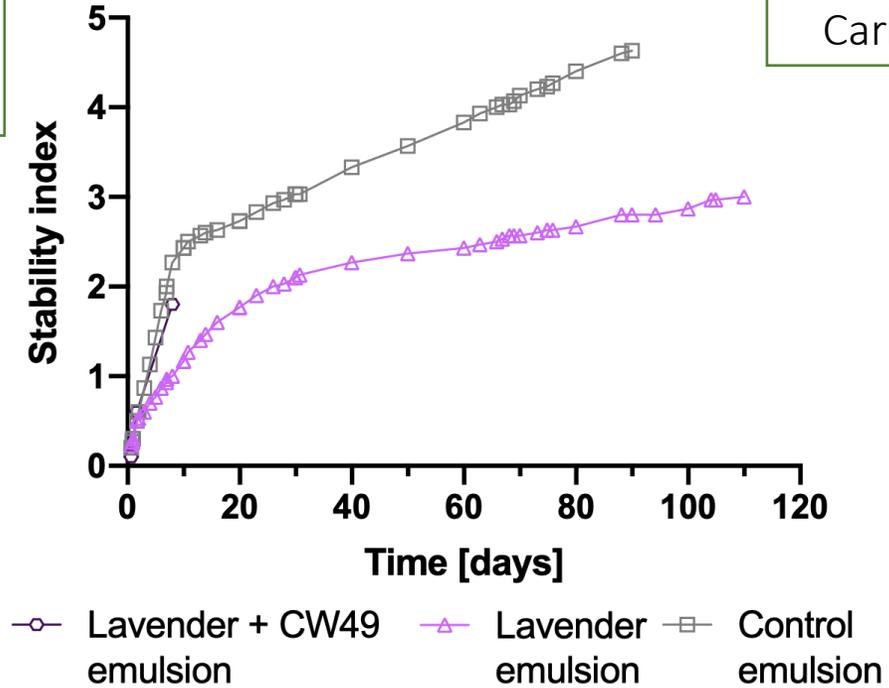
# Physicochemical Characterization

## Flow



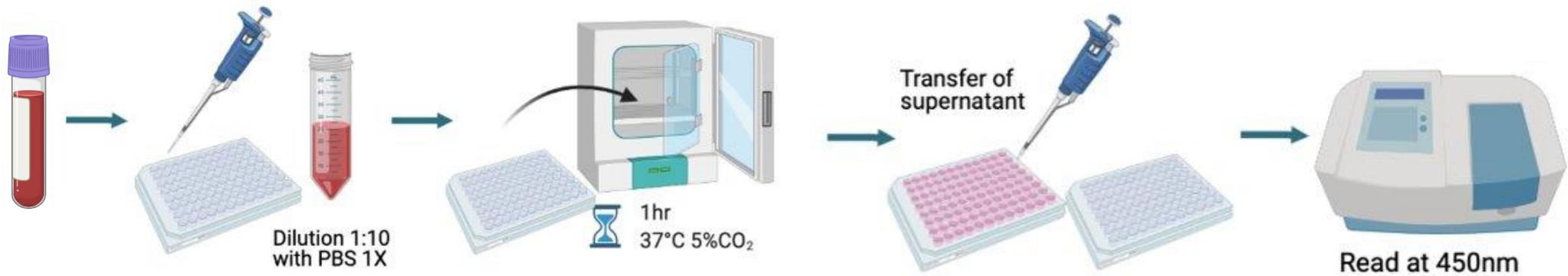
- Pseudo-plastic behavior
- Resistance to structural rupture during exposure to high shear rate
- Shear rate apparent viscosity

## Stability

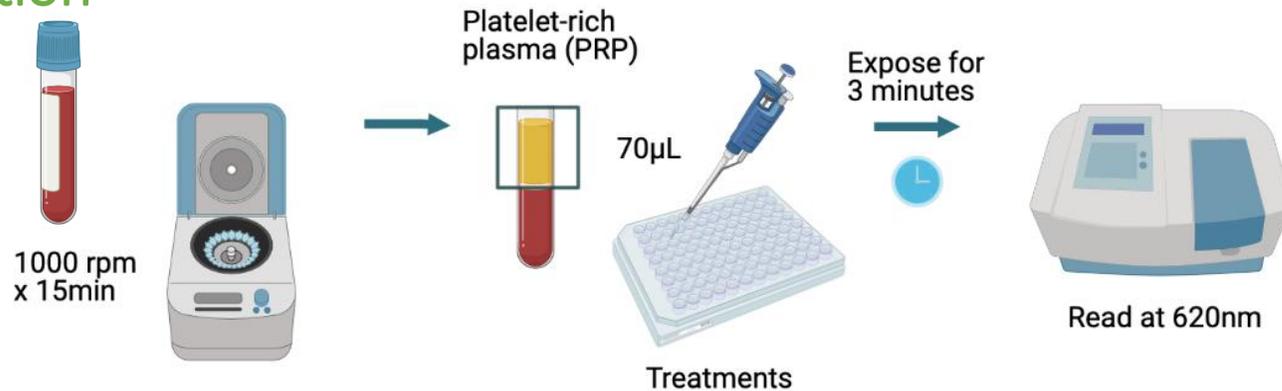


- Stable in time most likely due to the Carbopol

## Hemolysis



## Platelet activation

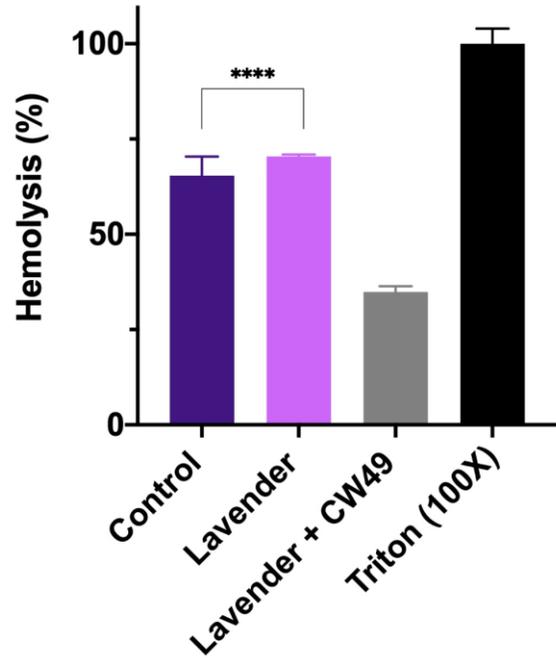


### Treatments:

- Control emulsion
- Lavender emulsion
- Lavender-CW49 emulsion

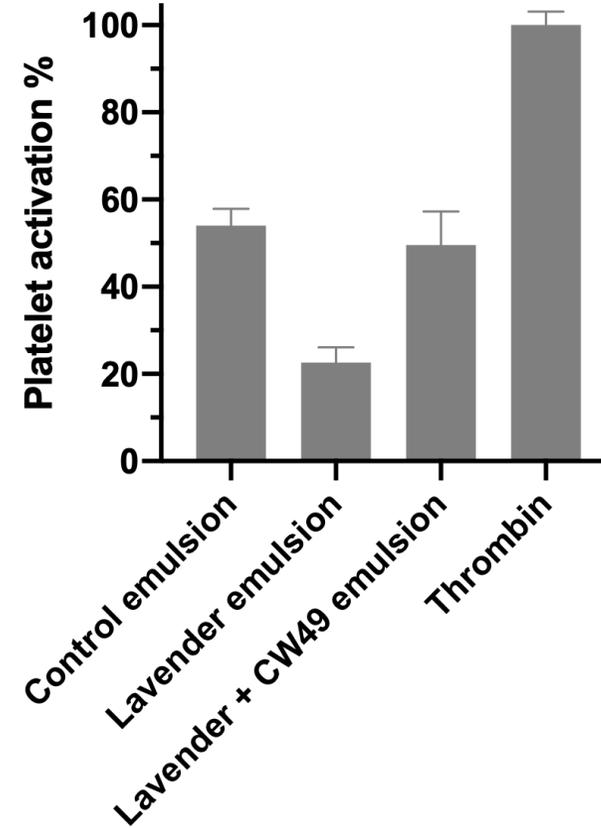
# Biocompatibility Assays

## Hemolysis



- Lavender emulsion is hemolytic
- By adding the peptide, the percentage of hemolysis decreases
- Emulsions are not intended for direct contact with human blood

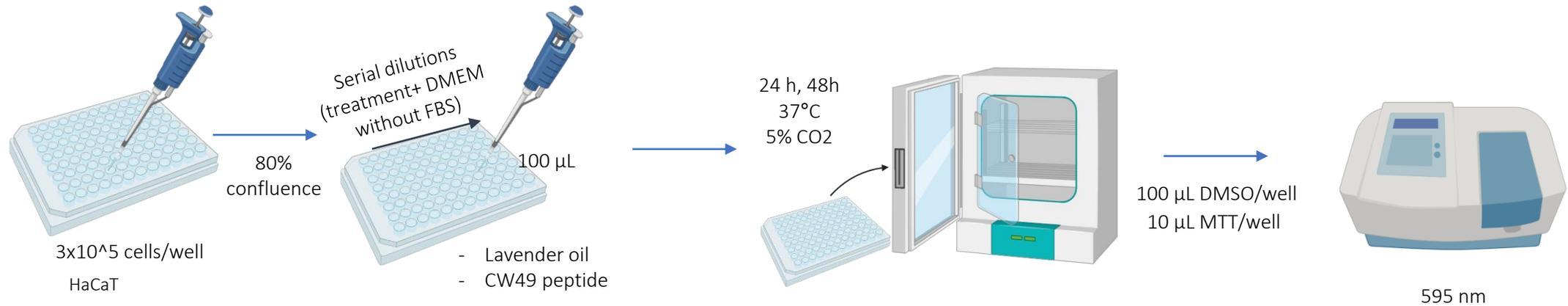
## Platelet activation



- Emulsions are platelet aggregators
- Promote formation of granular tissue in wounds

# Biocompatibility Assays

## Cell viability



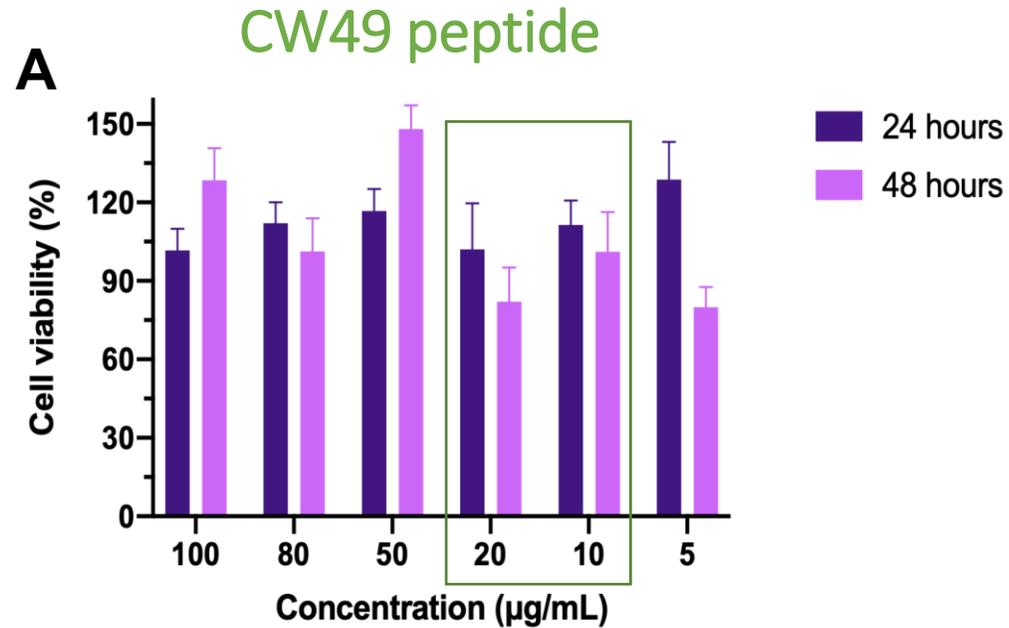
### Treatments:

- Lavender oil
- CW49 peptide

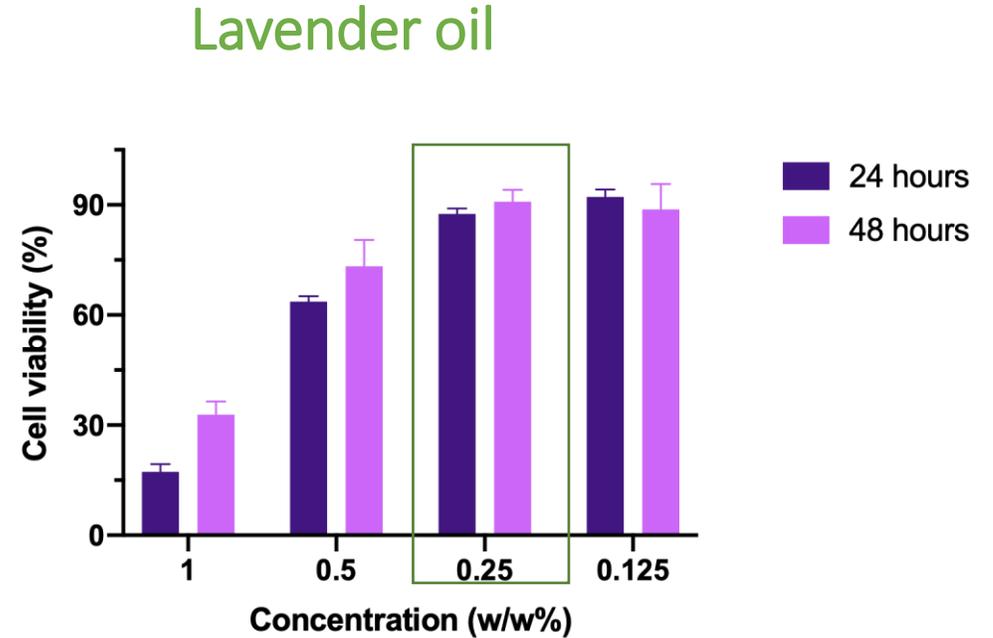
### Control:

- + DMEM
- DMSO

# Biocompatibility Assays



At 20 µg/mL and 10 µg/mL the viability decreased in about 15% and 5% after 48 hours.

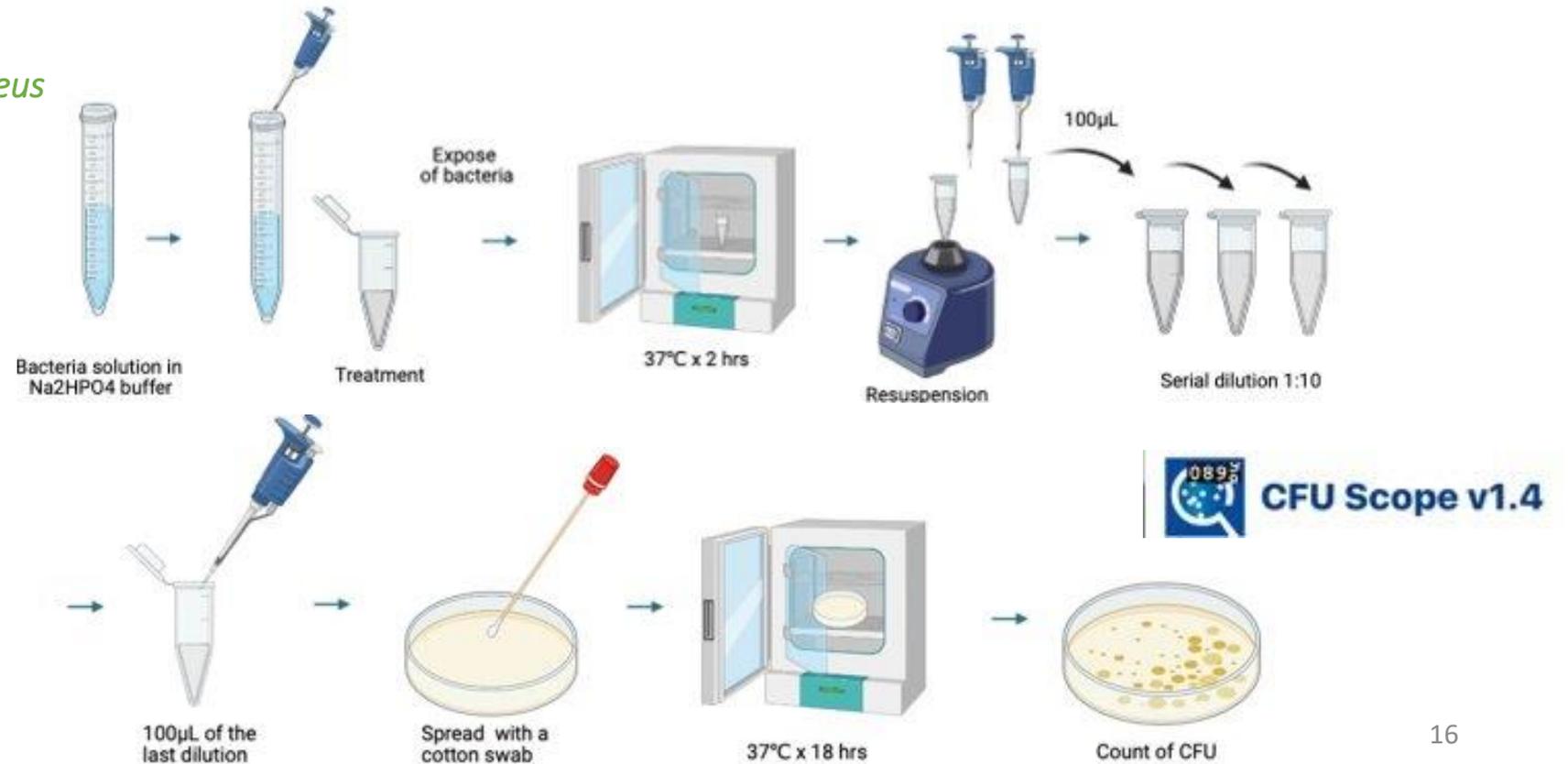


The 0.25%w/w concentration of lavender essential oil appears proliferative at 24 and 48 hours.

# Antibacterial Assays

ATCC 25922 *Escherichia coli*  
 ATCC 29213 *Staphylococcus aureus*

$1 \times 10^7$  CFU/mL

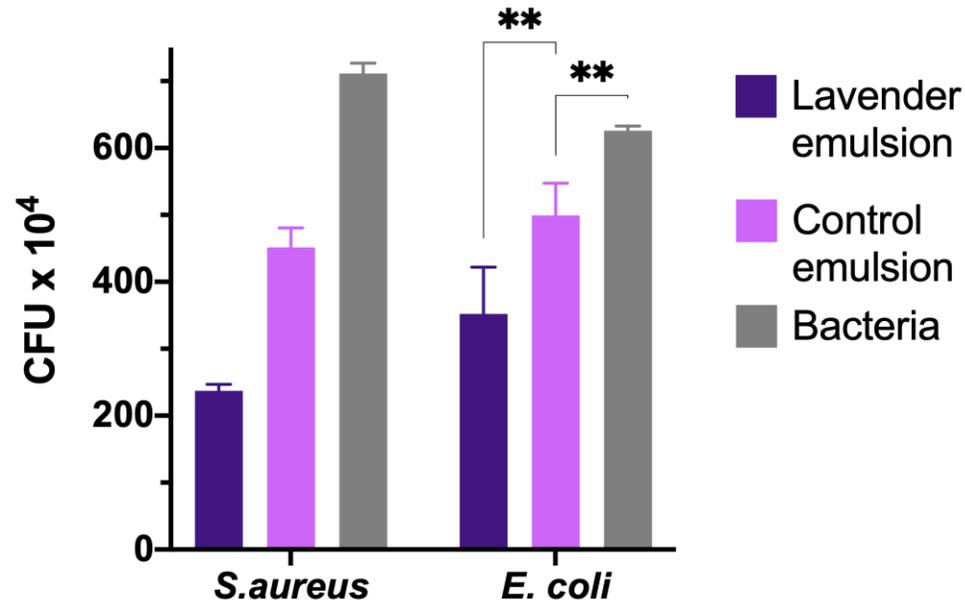


## Treatments:

- Control emulsion
- Lavender emulsion

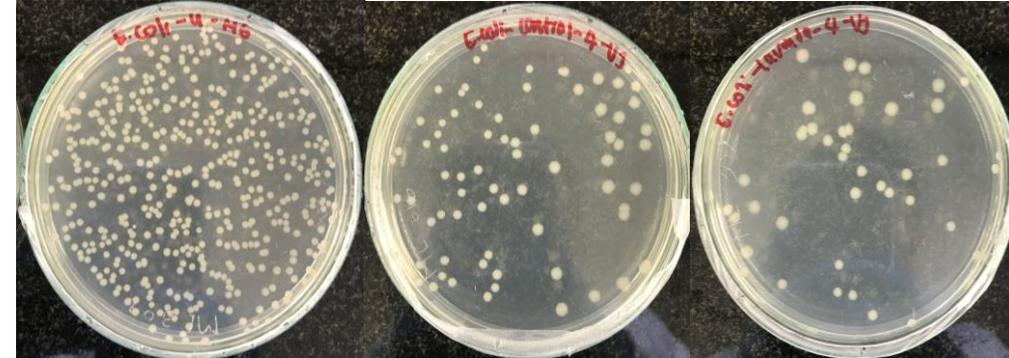
# Antibacterial Assays

## Bacterial Growth



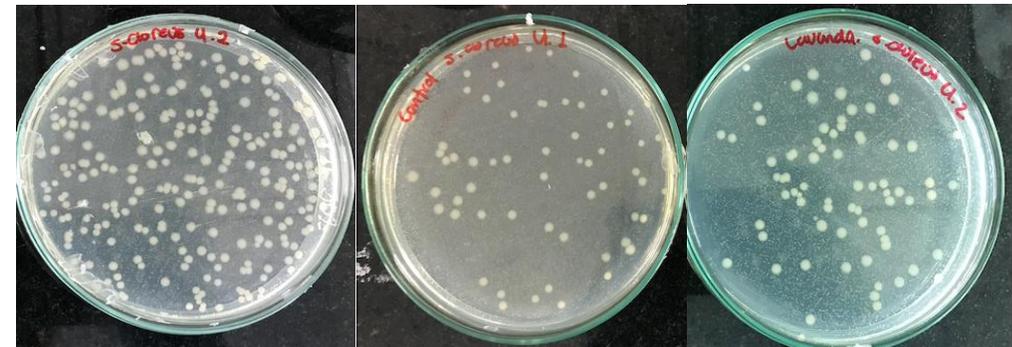
Lavender emulsion inhibits the growth of *E. coli* and *S. aureus*, thus exhibiting bacteriostatic behavior most likely due to the presence of the lavender essential oil.

### *E. coli*



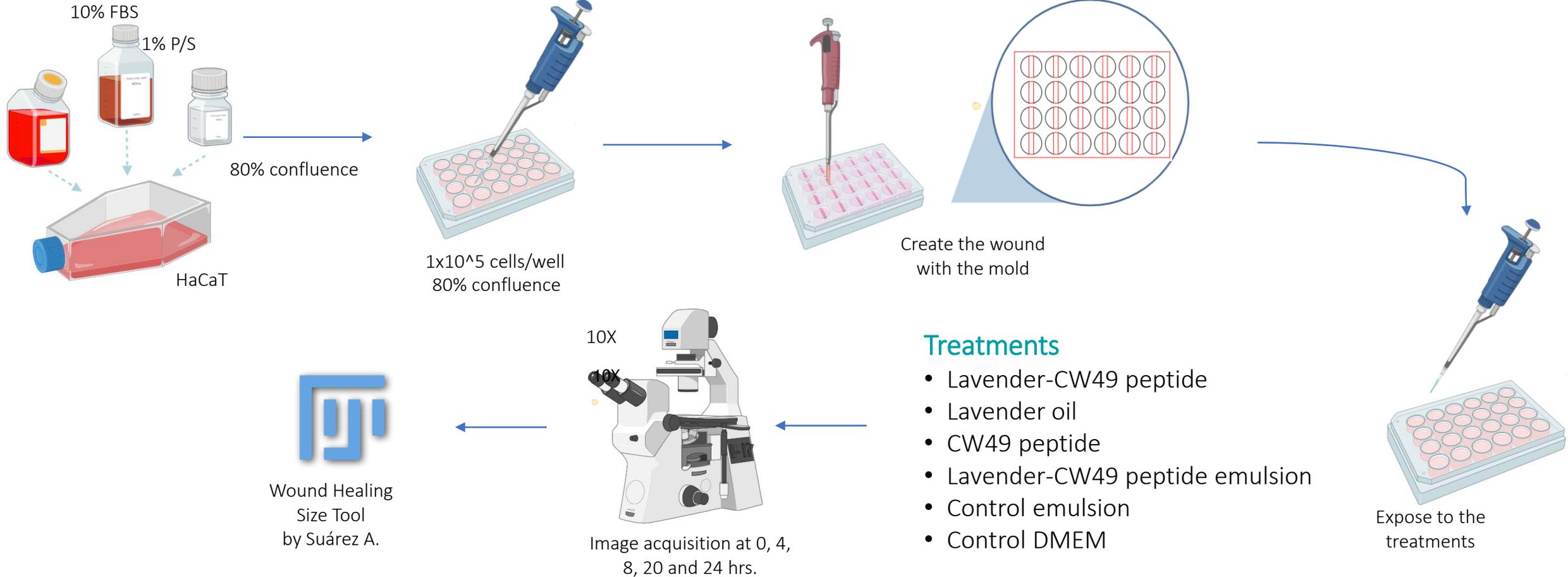
Control bacteria    Control emulsion    Lavender emulsion

### *S. aureus*



Control bacteria    Control emulsion    Lavender emulsion

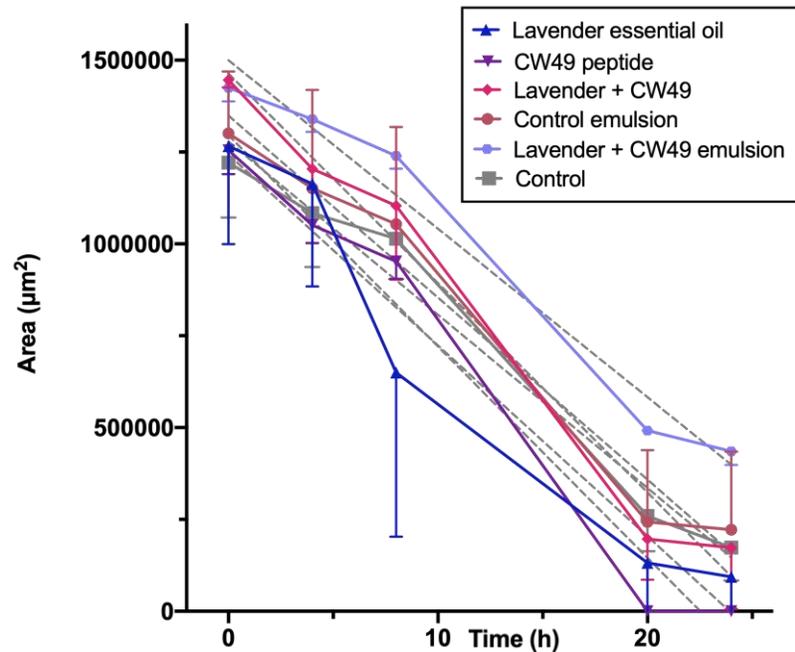
# Wound Healing Assay



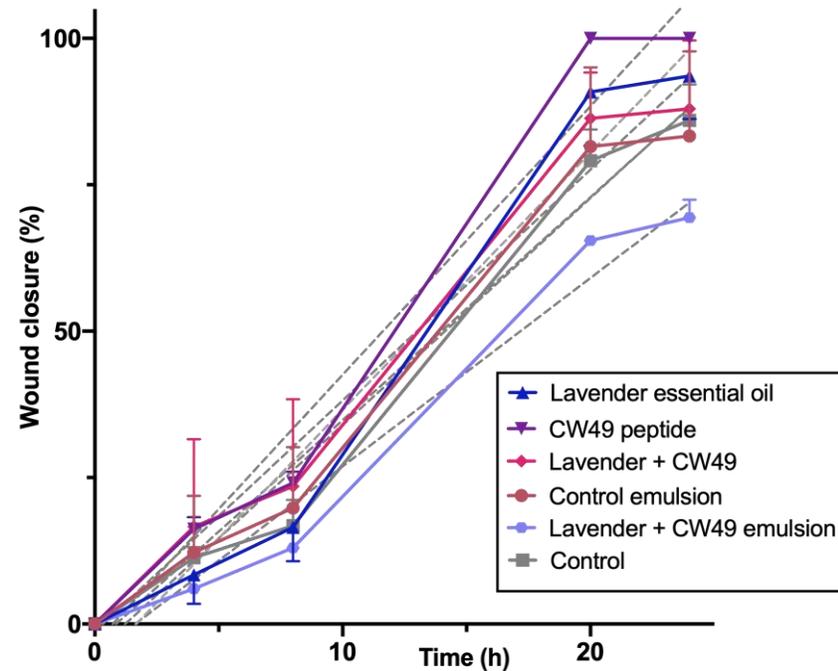
# Wound Healing Assay

- Together Lavender oil at 0.25%w/w and CW49 peptide at 20 $\mu$ g/mL reached almost 93% of closure at 24 hours.
- Each treatment separately reached almost 100% of closure at 20 hours.
- The wound closure exposed directly to the emulsions affect the healing most likely due to the cytotoxic effect of the surfactants.

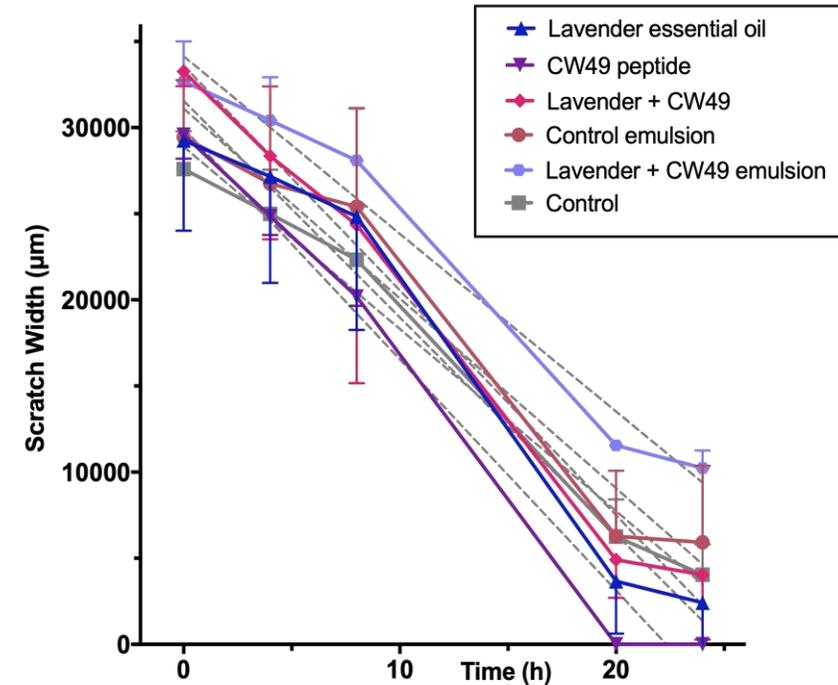
Area



Wound closure %



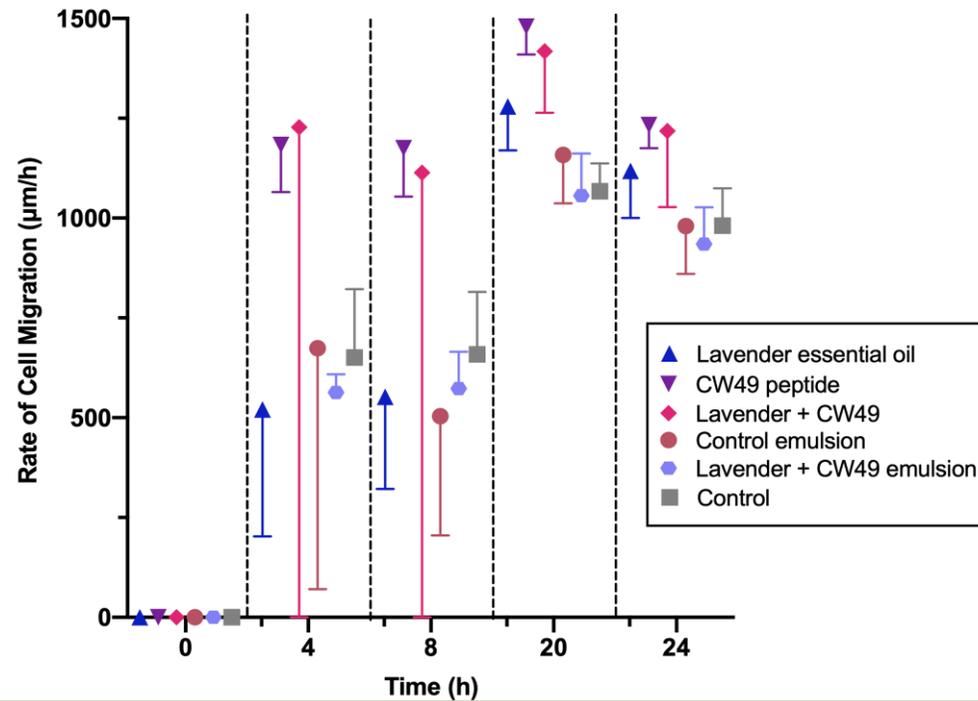
Scratch width



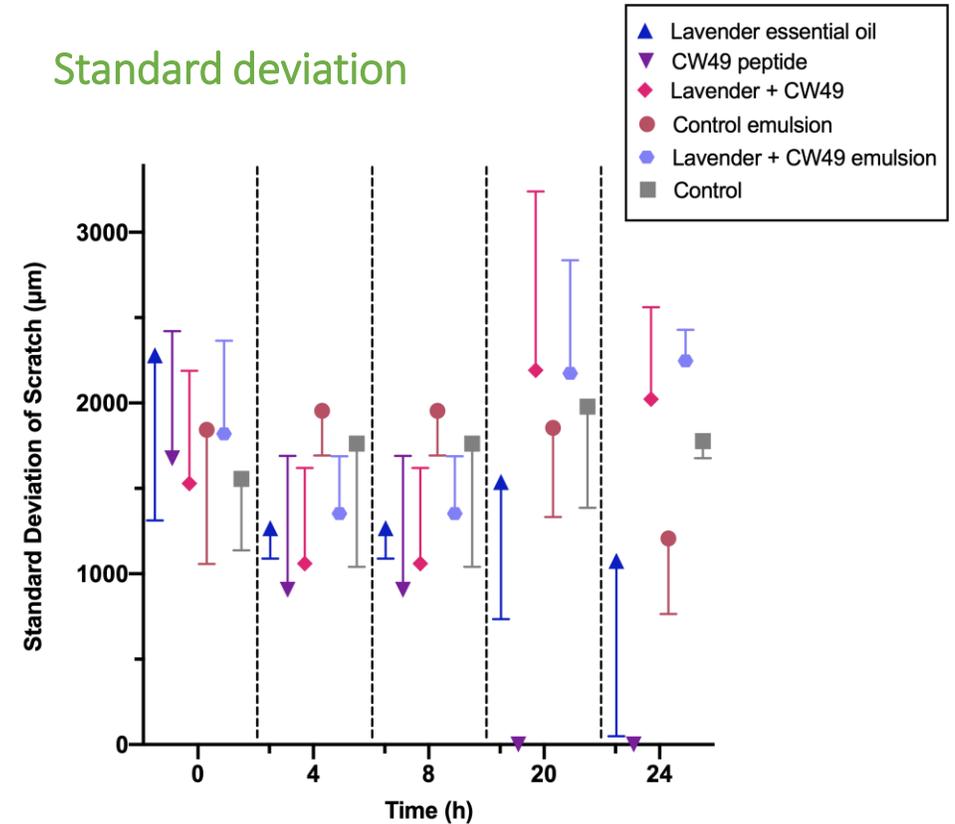
# Wound Healing Assay

- Deviation of the width appears not as function of the evaluated times or used medium.
- The rate of cell migration of cells exposed to the emulsions is slower.

### Rate of cell migration

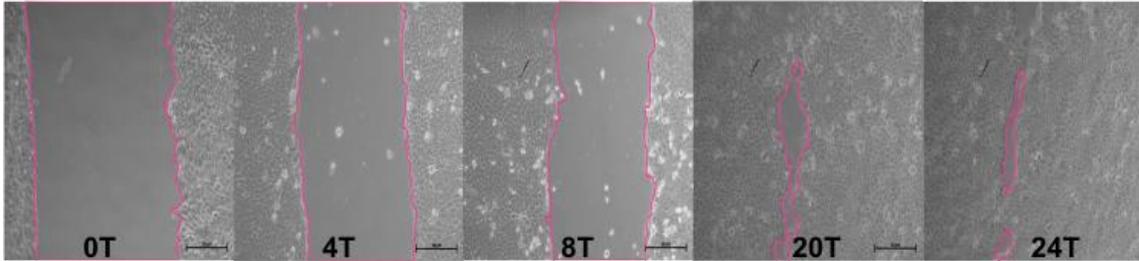


### Standard deviation

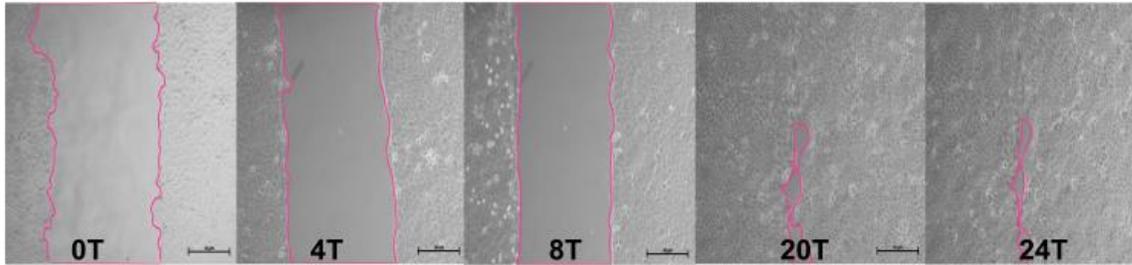


# Wound Healing Assay

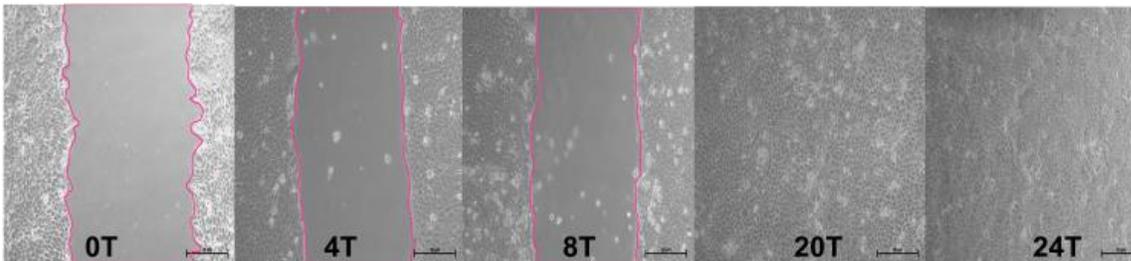
Lavender-peptide CW49



Lavender oil



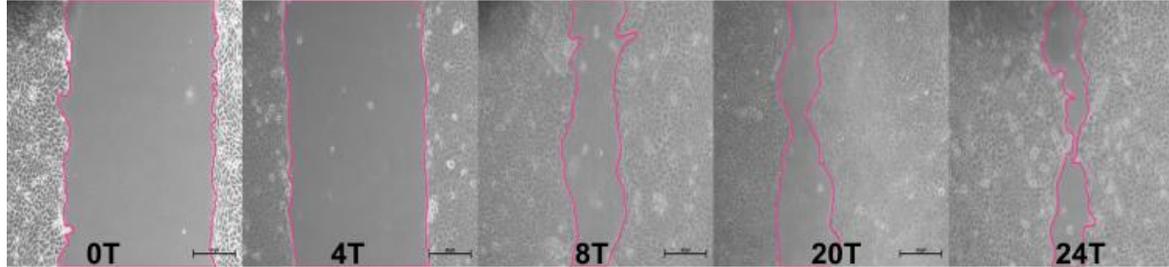
CW49 peptide



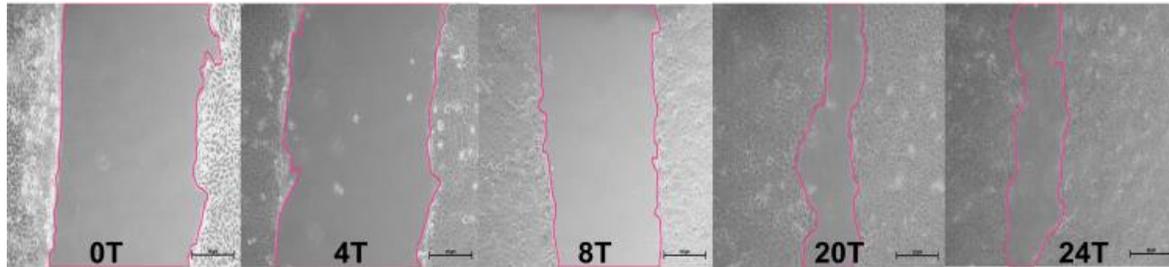
Wound closure reached ~94% for Lavender-CW49 peptide, ~95% for Lavender oil and ~99% for CW49 peptide

# Wound Healing Assay

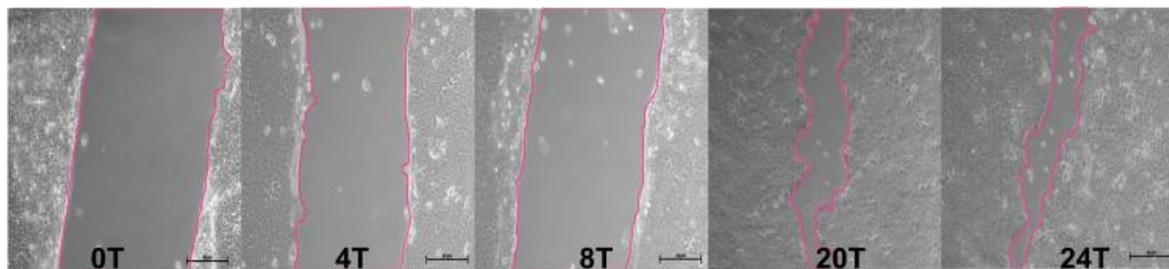
Lavender-CW49 peptide emulsion



Control emulsion



Control DMEM 10% FBS

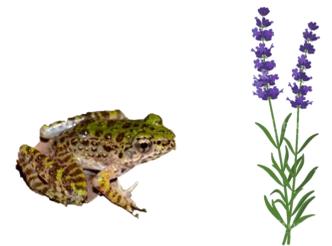


Wound closure reached ~ 65% for Lavender-CW49 peptide emulsion, ~75% for Control emulsion and ~75% for control DMEM 10%FBS

The emulsions are cytotoxic most likely due to the added surfactants

# Conclusions

- The emulsions showed a pseudoplastic fluid behavior, which is a critical attribute to assure skin retention and ease of application.
- The lavender emulsions are hemolytic and aggregating, but in the presence of the CW49 peptide, hemolysis is reduced significantly; however, platelet aggregation is beneficial for the wound healing process.
- The wound healing assays showed promising results for wound closure with the lavender-peptide extracts, which reached a **wound closure percentage of ~ 93% after 24 h.**
- The wound healing assays showed results for wound closure with the lavender-peptide emulsion, which reached a **wound closure percentage of 65% after 24 h.**
- Taken together, our findings indicate that the formulated emulsion might be considered as a potential wound healing topical treatment and will therefore undergo further testing *in vivo*.



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# THANK YOU FOR YOUR ATTENTION

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