# Multimodal stimulation system to control fibroblast proliferation using optical and ultrasonic stimulation

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# Outline

1. Introduction
2. Methods
3. Results and Discussions
4. Conclusions

# 1. Introduction

## Motivation

- The increased interest in personal skincare systems due to the aging population and the growing number of SNS users
- Low-cost active skin care systems are being developed, but side effects such as burns and skin coloration are increasing
- Optical stimulation using LED can cause different wavelength effects & Non-invasive ultrasonic stimulation induces rearrangement of skin and subcutaneous tissue collagen and elastic fibers
- Research on complex wavelengths or noninvasive fusion stimuli is insufficient and related research is required

## Goal

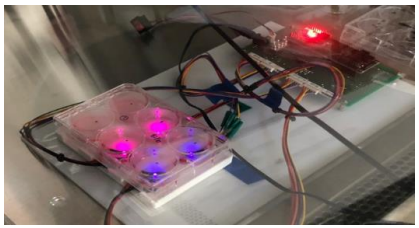
- Development of multimodal system that enables LED and ultrasonic simultaneous stimulation of a single/complex wavelength
- Development of the Skin Cell Activity Control System for Quantitative Measurement of Skin Cell Activity

# 2. Methods

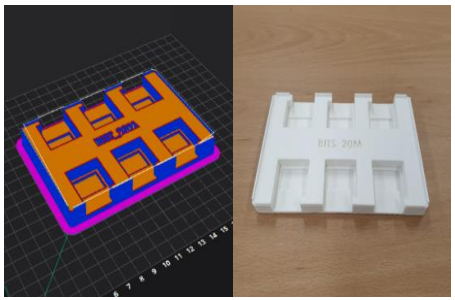
## Experimental Process

### Stimulation System

#### Multimodal System

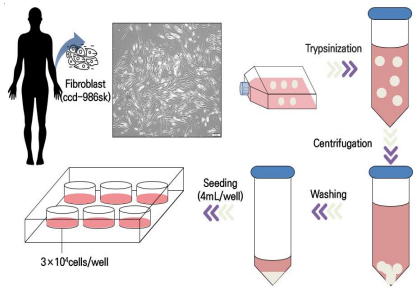


#### 3D Design

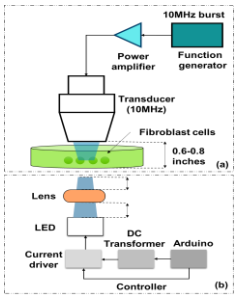


### *in-vitro* Experiment

#### Cell Preparation



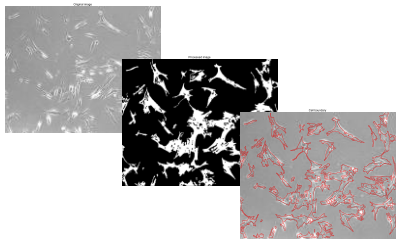

#### Multimodal Stimuli




### Quantitative Analysis

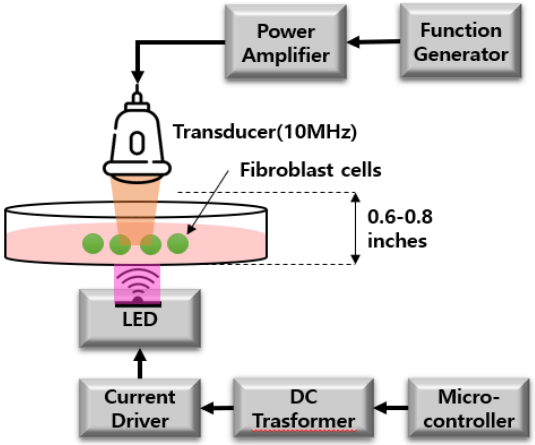
#### Image Acquisition

#### Image Quantification



# 2. Methods

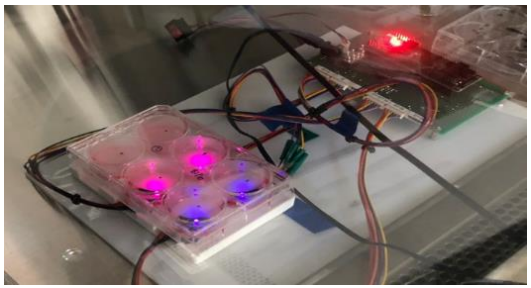
## Stimulation System




[Experimental diagram]

[Multimodal system]

[Ultrasonic system]



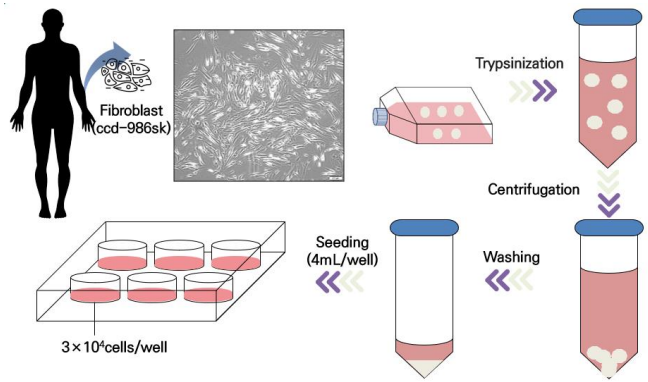
[LED system]

### Multimodal System

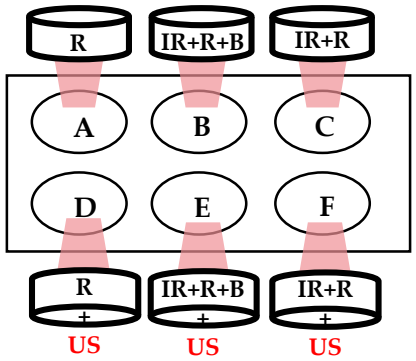
- **US (Ultrasonic) system**
  - 10MHz Olympus ultrasound probe, 20mVpp, 100cycle
- **LED (Optical) system**
  - Microcontroller (Atmega128)
  - LED (LG Innotek 3528, Blue (405~425), Red (620~640), IR (840~860) [nm], 3.5x 2.8x1.4 (LxWxH)[mm])
  - Radiant intensity (@10mA) : Blue (4mW/sr), Red (3.5mW/sr), IR (2.2mW/sr)

# 2. Methods

## *in-vitro* Experiment




[Cell preparation process]



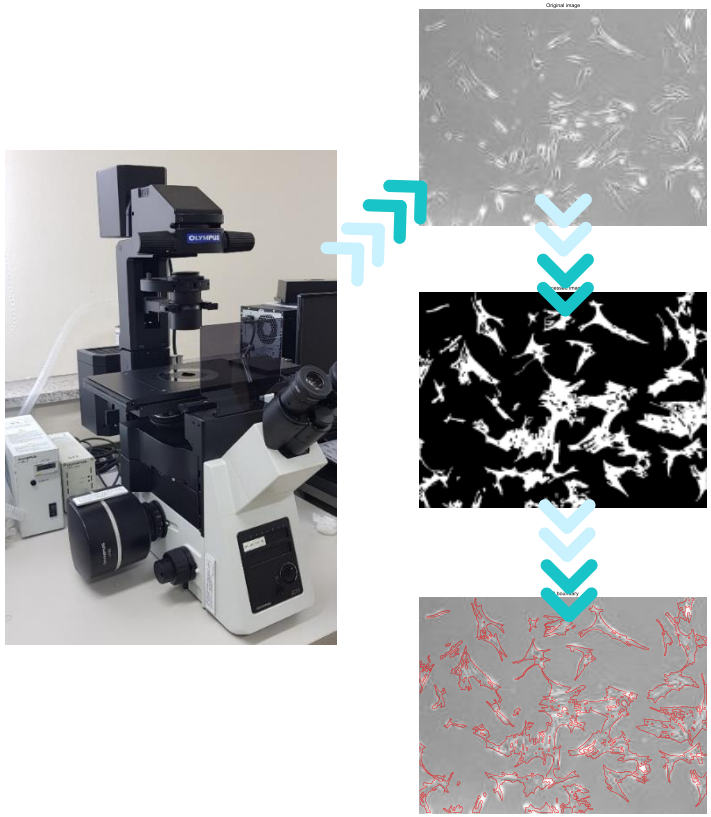

[Stimulation type of each group]

### Cell preparation

- **Cell information**
  - Human skin fibroblast cells (CCD-986 sk, 22yrs black female)
  - 3 x $10^4$ cells/well x 6 wells / flask
- **Multimodal stimulation experiment**
  - Stimulation applied for 30 minutes every day for a total of 6 days.(control n=12)
  - LED (n=9): GroupA (R), GroupB (IR+R+B), GroupC (IR+R)
  - LED + Ultrasonic (US) (n=9): GroupD (R+US), GroupE (IR+R+B+US), GroupF (IR+R+US)

# 2. Methods

## Quantitative Analysis



[Acquisition system and image processing progress]

### Image Acquisition

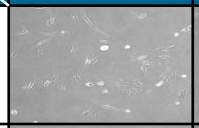
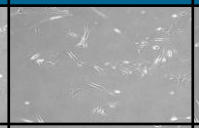
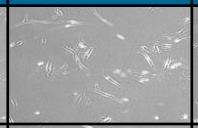
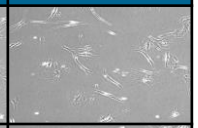
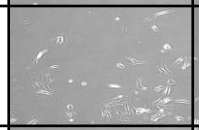
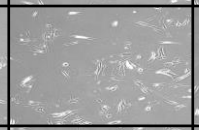
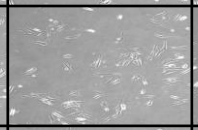
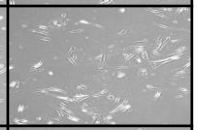
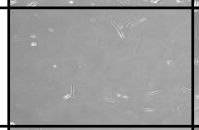
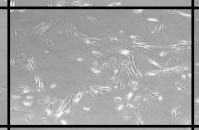
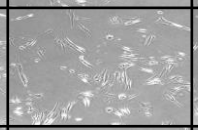
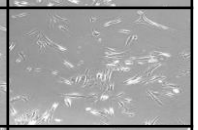
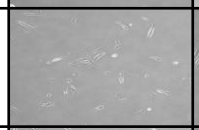
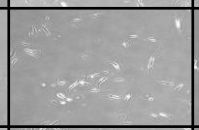
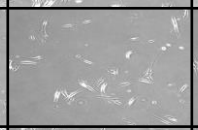
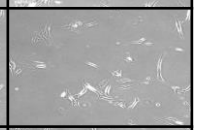
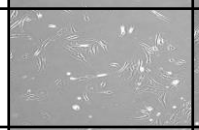
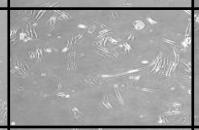
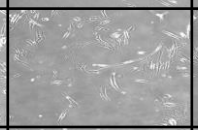
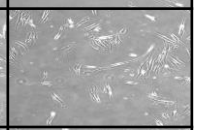
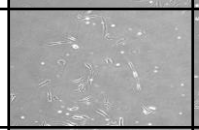
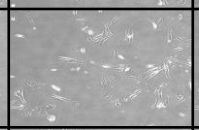
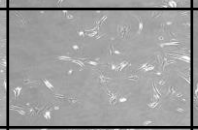
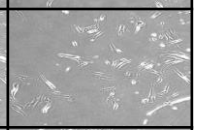
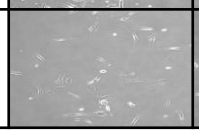
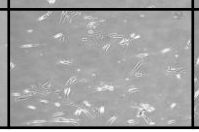
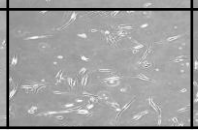
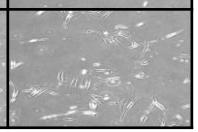
- **Image acquisition system**
  - Inverted fluorescent microscope (IX73, Olympus, Japan)
  - Camera (DP80 Dual Sensor monochrome and color camera, Olympus, Japan)

### Image Quantification

- **Processing program**
  - Matlab (Mathworks, USA)
  - Image acquisition → Image processing → Cell area quantification → Statistical analysis

# 3. Results

## Representative Images

| Day / Group | Day1 | Day3 | Day5 | Day6 |
|---|---|---|---|---|
| Control |  |  |  |  |
| Group A (R) |  |  |  |  |
| Group B (IR+R+B) |  |  |  |  |
| Group C (IR+R) |  |  |  |  |
| Group D (R+US) |  |  |  |  |
| Group E (IR+R+B + US) |  |  |  |  |
| Group F (IR+R + US) |  |  |  |  |

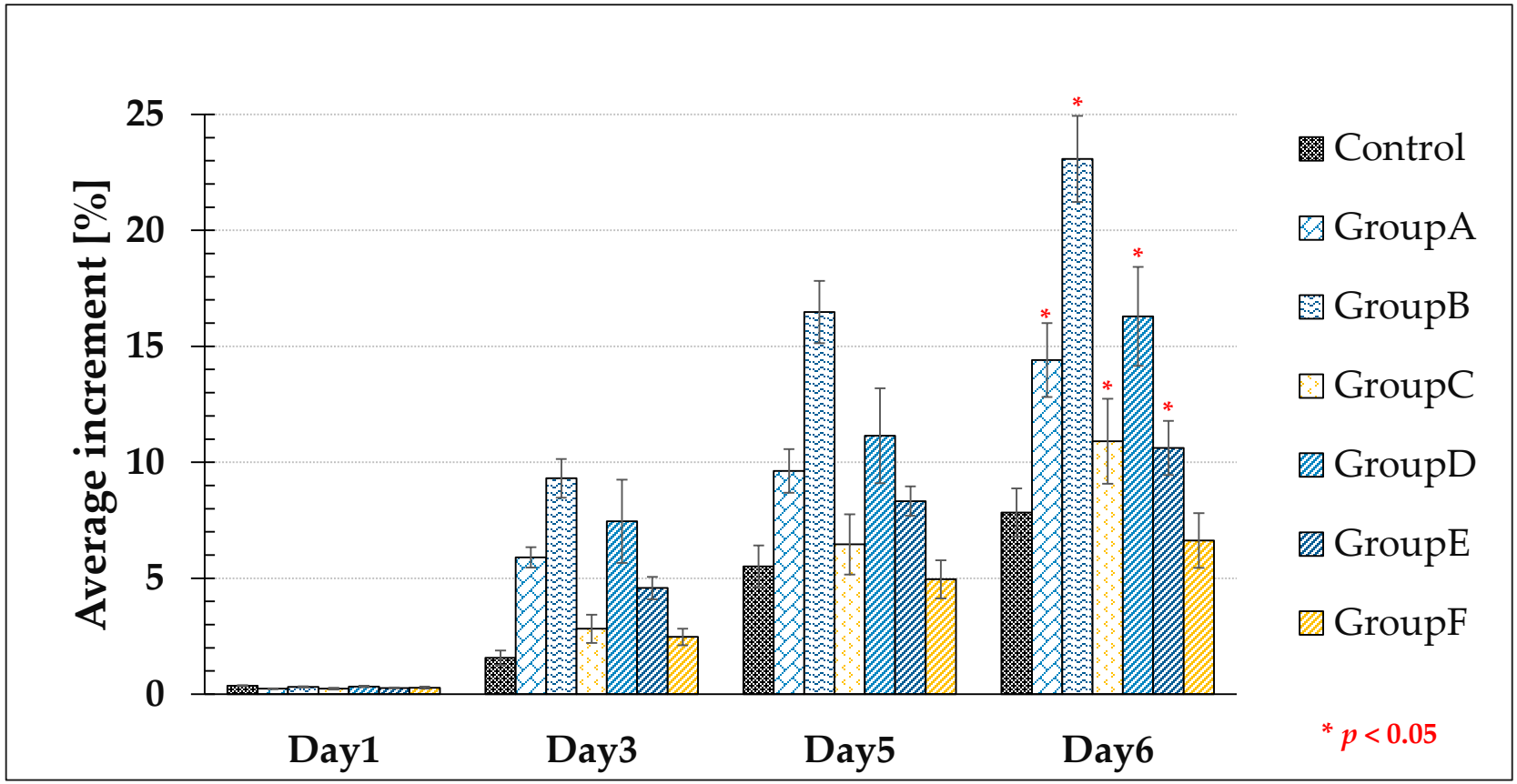
[Representative images of fibroblast cells per group]

| Average [%] | Day1 | Day3 | Day5 | Day6 |
|---|---|---|---|---|
| Control | 0.37±0.03 | 1.57±0.31 | 5.51±0.90 | 7.83±1.04 |
| Group A | 0.23±0.02 | 5.90±0.44 | 9.63±0.94 | 14.41±1.59 |
| Group B | 0.32±0.03 | 9.31±0.83 | 16.48±1.34 | 23.08±1.86 |
| Group C | 0.23±0.04 | 2.82±0.60 | 6.46±1.30 | 10.91±1.83 |
| Group D | 0.33±0.03 | 7.45±1.79 | 11.14±2.05 | 16.29±2.13 |
| Group E | 0.27±0.02 | 4.58±0.48 | 8.33±0.63 | 10.62±1.16 |
| Group F | 0.28±0.04 | 2.47±0.36 | 4.96±0.82 | 6.63±1.18 |

[Increment of average cell densities for each group]

# 3. Results

## Cell densities for each group



# 4. Conclusions



- In this study, an activity control system was developed that enables selective stimulation of LED and non-invasive ultrasound stimuli in single/composite wavelength bands.
- As a result , the LED of the R showed a higher growth rate than the combined LED of IR+R+B, but a lower than the combined wavelength band IR+R+B.

  (Group B (IR+R+B) > Group A (R) > Group C (IR+R))
- On the other hand, for multimodal stimuli are applied, R+US stimuli shows a higher growth rate than other stimuli.

  (Group D (R+US) > Group E (IR+R+B+US) > Group F (IR+R+US)))
- The results show that multi LED stimuli, including blue wavelengths, are more effective than single wavelength stimuli and ultrasonic added stimuli has the highest cell growth rate with red wavelength bands.

# Thank you

Q & A