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Title: Investigation of a Blue Light LED Device to Suppress Wound Pathogens Using a Collagen-Based Synthetic Skin Model

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes.**

***Videographer:** Please film computer screen as close up shot for shots labeled as SCREEN (5.5.2, 5.8.1, and 5.9.1)*

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **YES**

Authors' NOTE: We will provide the details once the confirmation is received.

Current Protocol Length

Number of Steps: 29

Number of Shots: 43

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**. Also, the statements have been edited for length and clarity.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Siyam Subair**: Our research investigates how 405-nanometer blue light can safely suppress wound pathogens using a collagen-based synthetic skin model.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Natasha Singh**: Most studies use broth cultures, agar plates, or animal models; we introduce a realistic, reproducible skin-mimicking platform for phototherapy testing.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Mohamed Hasanar**: This method evaluates phototherapy light sources by testing antimicrobial efficacy, while preserving collagen integrity and safety on skin-like surfaces.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Pratibha Sharma**: The key challenge in this protocol is maintaining uniform irradiance and accurate fluence control of the light sources to ensure reproducible antimicrobial results.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Siyam Subair:** This protocol can be expanded to biofilm testing, oxidative stress analysis, next-generation light-device optimization, and screening of similar novel therapeutic interventions.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What is the key takeaway you hope viewers remember after watching your video article?

1.6. **Enter author name**, **Enter author title**: (authors will present their testimonial statements live)

1.6.1: INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

What value do you expect this video article to have for other researchers working in your field?

1.7. **Enter author name**, **Enter author title**: (authors will present their testimonial statements live)

1.7.1: INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Culture Preparation, Skin Model Inoculation, and Irradiation with Blue and UV-C Light

Demonstrators: Siyam Subair, Pratibha Sharma

- 2.1. To begin, obtain the desired bacterial [1] and fungal strains [2]. Pick a single isolated colony and aseptically inoculate it into 5 milliliters of Tryptic Soy Broth [3]. Once all strains are inoculated, incubate the cultures [4-TXT].
 - 2.1.1. WIDE: Talent placing the bacterial and fungal culture plates on the sterile workbench. Authors: Please ensure the strains are appropriately labeled.
Videographer: Obtain a CU shot of this as well. **Videographer's NOTE: 2.1.1 – take 5 is WS, take 6 is CU**
 - 2.1.2. TEXT ON A PLAIN BACKGROUND:
Strains used:
Staphylococcus aureus (ATCC 25923)
Escherichia coli (ATCC 25922)
Enterobacter aerogenes (ATCC 13048)
Pseudomonas aeruginosa (ATCC 27853)
Acinetobacter baumannii (ATCC 19606)
Klebsiella pneumoniae (ATCC 13883)
Candida albicans (ATCC 10231)
 - 2.1.3. Talent picking a single isolated colony aseptically and inoculating it into 5 ml of Tryptic Soy Broth.
 - 2.1.4. Talent placing the cultures in the incubator. **TXT: Bacteria: - 35 °C, 24 h; Fungi: 22 °C, 5 days**
- 2.2. After the desired incubation time, using sterile PBS (*P-B-S*), adjust the turbidity to the 0.5 McFarland Standard for antimicrobial assays [1-TXT].
 - 2.2.1. Talent adjusting turbidity using sterile PBS. **TXT: Desired Turbidity: 1.5×10^8 CFU/mL** **Videographer's NOTE: take 1 WS, take 2 CU**
- 2.3. Next, take previously prepared hydrated synthetic skin sections [1] and inoculate each section with 100 microliters of microbial suspension [2]. Using a sterile spreader, distribute the suspension evenly across the surface [3].

- 2.3.1. Talent placing the hydrated synthetic skin sample on sterile workbench.
- 2.3.2. Talent inoculating the skin section with 100 μ L microbial suspension. **NOTE:** 2.3.2 and 2.3.3 are filmed in a single shot
- 2.3.3. Talent spreading the suspension using a sterile spreader.
- 2.4. Then, air dry the inoculated samples in a sterile chamber under ambient conditions [1-TXT].
 - 2.4.1. Shot of the samples plates getting dried in the sterile chamber. **TXT: Temperature: 22 - 24 °C; Relative Humidity: 60% - 70%; Time: 15 min**
- 2.5. Assign the samples to three treatment groups for varying light exposure [1-TXT].
 - 2.5.1. Talent sorting the samples plates into 3 groups. **Authors: Please ensure the treatment group details are visible. Put labels on a sterile workbench if possible before sorting the samples. TXT: Group A: Untreated control; Group B: Blue light LED (405 nm); Group C: UV-C LED (265 nm)**
- 2.6. For irradiation, mount the blue-light LED (*L-E-D*) device onto an aluminum heat sink [1].
 - 2.6.1. Shot of the blue-light LED device mounted onto an aluminum heat sink.
- 2.7. Use a calibrated optical power meter to measure irradiance at the exact treatment plane [1]. Adjust the probe height to account for sensor thickness [2].
 - 2.7.1. Shot of a calibrated optical power meter measuring irradiance at the exact treatment plane.
 - 2.7.2. Talent adjusting the probe height.
- 2.8. Now, adjust the throw distance of the LED to standardize the working irradiance [1-TXT].
 - 2.8.1. Talent adjusting the throw distance of the LED to standardize the working irradiance. **TXT: Working irradiance: 30 mW/cm²**
- 2.9. Place the inoculated skin swatches from Group B aseptically into sterile Petri dishes without lids [1]. Position the dishes directly under the blue-light LED for irradiation [2-TXT]. Similarly, irradiate Group C under UV-C (*U-V-C*) light [3].
 - 2.9.1. Talent placing the inoculated skin swatches aseptically into sterile Petri dishes without lids. **NOTE: 2.9.1 and 2.9.2 are filmed in a single shot**

- 2.9.2. Talent positioning Group B dishes directly under the blue-light LED and light is turned on. **TXT: Fluence: 27 J/cm², 15 min**
- 2.9.3. Shot of Group C plate under UV-C light

3. Antimicrobial Efficacy of Blue Light and UV-C Irradiation Against Pathogens

Demonstrators: Natasha Singh

- 3.1. Transfer the treated synthetic skin swatch into a sterile 15-milliter tube containing 9 milliliters of PBS at pH (P-H) 7.4 [1] and vortex it gently to dislodge surface-adherent microbes [2].
 - 3.1.1. Talent transferring the treated skin swatch into a sterile 15 mL tube containing 9 ml PBS. **NOTE: 3.1.1 and 3.1.2 are filmed in a single shot**
 - 3.1.2. Talent gently vortexing the tube.
- 3.2. Now, prepare ten-fold serial dilutions of the suspension [1].
 - 3.2.1. Representative shot of talent preparing serial ten-fold dilutions of the suspension. **Authors: Please have the dilutions ready and for demonstration show the last fold dilution once.**
- 3.3. Plate 100 microliters from the selected dilutions onto the agar plates [1] and incubate the plates [2].
 - 3.3.1. Talent plating 100 µL from the selected onto the agar plates. **TXT: Bacteria - Tryptic Soy Agar, Fungi - Sabouraud Dextrose Agar**
 - 3.3.2. Talent transferring the plates to incubators set to the required conditions.
- 3.4. At the end of the incubation period, count the number of colonies [1] and record the number of colony-forming units [2].
 - 3.4.1. Talent using the semi-automated colony counter for counting the colonies **NOTE: 3.4.1 and 3.4.2 are filmed in a single shot**
 - 3.4.2. Talent documenting the number of colony forming units.
- 3.5. Calculate microbial reduction using the presented equations [1].
 - 3.5.1. TEXT ON A PLAIN BACKGROUND:
Log Reduction = $\log_{10}(A) - \log_{10}(B)$
Percent Reduction = $((A - B)/A) \times 100$

Where A = CFU count from control; B = CFU count from treated samples

4. Assessment of Blue Light in a Simulated Airborne Contamination Model

Demonstrators: Siyam Subair, Natasha Singh

- 4.1. To simulate nosocomial airborne exposure, use a customized acrylic aerosol chamber under controlled environmental conditions [1].

- 4.1.1. WIDE: The acrylic aerosol chamber setup.

- 4.2. Place sterile agar plates inside the chamber to monitor microbial fall-out activity [1].

- 4.2.1. Talent positioning agar plates and opening it inside the chamber. **NOTE: take 3 WS, take 4 CU**

- 4.3. Then, position the sterile skin swatches inside the chamber [1].

- 4.3.1. Talent arranging synthetic skin swatches.

- 4.4. Using a nebulizer, aerosolize 2 milliliters of microbial suspension into the chamber [1].

- 4.4.1. Microbial suspension is nebulized into the sealed chamber.

- 4.5. Now, expose the swatches to continuous blue light illumination from 20 centimeters for 30 minutes [1-TXT].

- 4.5.1. Talent exposing the swatches to continuous blue light illumination in the chamber. **TXT: Blue light exposure: 10 $\mu\text{W}/\text{cm}^2$**

- 4.6. For the control, repeat identical chamber conditions with the blue light device turned off [1]. Quantify viable counts from the swatches by standard plate count analysis [2-TXT].

- 4.6.1. Shot of plates, skin swatches inside the chamber being aerosolized with the blue light turned off. **NOTE: Includes CU of meter reading and spray**

- 4.6.2. Representative shot of talent performing standard plate count analysis **TXT: Perform optical transmission and embedded-colony testing**

5. ATR-FTIR Analysis of Synthetic Skin Following Blue Light and UV-C Irradiation

Demonstrator: Mohamed Hasanar

- 5.1. For FTIR (*F-T-I-R*) analysis, obtain synthetic skin swatches and hydrate them as per the manufacturer's instructions [1]. Divide the hydrated swatches into four sets on a sterile surface [2].
 - 5.1.1. Talent hydrating the skin swatches.
 - 5.1.2. Shot of swatches arranged flat on a sterile surface for irradiation.
- 5.2. Expose two sets of swatches to blue light, one for 15 minutes and the other for 60 minutes [1].
 - 5.2.1. Show blue light illuminating the swatches. **TXT: Exposure: 405 nm, 30 mW/cm²**
- 5.3. Similarly, expose two sets of swatches to UV-C light, one for 15 minutes, and the other for 60 minutes [1].
 - 5.3.1. Show UV-C light irradiating the swatches. **TXT: Exposure: 265 nm, 3 mW/cm²**
- 5.4. After irradiation, gently blot the samples to remove surface moisture [1].
 - 5.4.1. Talent carefully blotting swatches with lint-free tissue.
- 5.5. Analyze the samples using FTIR spectroscopy [1] to assess chemical and structural changes [2].
 - 5.5.1. WIDE: Shot of the FT-IR instrument connected to a computer workstation.
 - 5.5.2. SCREEN: Computer display showing the spectrum acquisition *Videographer: Please film computer screen for this shot as a close-up shot.*
- 5.6. Configure the FTIR instrument in ATR mode and prepare the crystal surface for sample placement [1].
 - 5.6.1. Close-up of the clean ATR crystal before sample placement.
- 5.7. Place the swatch onto the ATR (*A-T-R*) crystal for spectral acquisition [1].
 - 5.7.1. Close-up of the swatch placed on the ATR iD7 crystal, and the pressure arm engaged.
- 5.8. Perform direct spectral comparisons between untreated control and irradiated samples [1].
 - 5.8.1. SCREEN: Overlaid spectra of control and irradiated samples displayed in the analysis software. *Videographer: Please film computer screen for this shot as a close-up shot.* NOTE: For the FT-IR analysis, steps 5.8.1 and 5.9.1 were captured as single frames (not video), in accordance with the script.

- 5.9. Next, use the untreated control swatch as a background reference and scan the irradiated samples to obtain baseline-subtracted spectra [1].
- 5.9.1. SCREEN: in the analysis software, control is selected as a reference and then irradiated samples are scanned to obtain baseline-subtracted spectra.
Videographer: Please film computer screen for this shot as a close-up shot.

Results

6. Results

- 6.1. Average log reductions reveal species-dependent differences in susceptibility [1], with *Klebsiella pneumoniae* demonstrating the greatest sensitivity to blue light [2] and *Staphylococcus aureus* showing comparatively lower, though still significant, reduction [3]. UV-C irradiation achieved higher microbial reductions across all tested species [4].
 - 6.1.1. LAB MEDIA: Figure 5
 - 6.1.2. LAB MEDIA: Figure 5 *Video Editor: Emphasize the blue bar for “Klebsiella pneumoniae”*
 - 6.1.3. LAB MEDIA: Figure 5 *Video Editor: Emphasize the blue bar for “Staphylococcus aureus”*
 - 6.1.4. LAB MEDIA: Figure 5 *Video Editor: Emphasize all the orange bars*
- 6.2. Blue light and UV-C irradiation demonstrated broad-spectrum antimicrobial activity against clinically relevant bacterial pathogens [1], including *Escherichia coli* [2], *Pseudomonas aeruginosa* [3], *Staphylococcus aureus* [4], and *Candida albicans* [5].
 - 6.2.1. LAB MEDIA: Figure 6
 - 6.2.2. LAB MEDIA: Figure 6A *Video editor: Please emphasize BL Treated and UV Treated image panels.*
 - 6.2.3. LAB MEDIA: Figure 6C *Video editor: Please emphasize BL Treated and UV Treated image panels.*
 - 6.2.4. LAB MEDIA: Figure 6D *Video editor: Please emphasize BL Treated and UV Treated image panels.*
 - 6.2.5. LAB MEDIA: Figure 6B *Video editor: Please emphasize BL Treated and UV Treated image panels.*
- 6.3. Exposure to blue light caused a time-dependent reduction in all three wound pathogens [1], with *Escherichia coli* and *Candida albicans* inactivating rapidly [2] and *Staphylococcus aureus* showing a slower, progressive decline [3].
 - 6.3.1. LAB MEDIA: Figure 7
 - 6.3.2. LAB MEDIA: Figure 7 *Video Editor: Please emphasize the blue and green lines in the graph.*
 - 6.3.3. LAB MEDIA: Figure 7 *Video Editor: Please emphasize the orange line in the graph.*

- 6.4. Blue light efficacy was further assessed under simulated airborne contamination conditions [1]. Continuous exposure significantly decreased microbial deposition on synthetic skin surfaces [2] relative to untreated controls for every species evaluated [3].
- 6.4.1. LAB MEDIA: Figure 8
- 6.4.2. LAB MEDIA: Figure 8 *Video Editor: Please emphasize all the blue bars in the graph.*
- 6.4.3. LAB MEDIA: Figure 8 *Video Editor: Please emphasize all the orange bars in the graph.*
- 6.5. Baseline-corrected ATR-FTIR spectra indicated that UV-C treatment reduced the intensity of amide peaks and enhanced Carbon-Oxygen stretching vibrations, reflecting collagen breakdown [1]. In contrast, blue-light exposure did not induce any significant spectral or chemical changes [2].
- 6.5.1. LAB MEDIA: Figure 11 *Video Editor: Please emphasize the graph labeled 'UV 1hour - control'*
- 6.5.2. LAB MEDIA: Figure 11 *Video Editor: Please emphasize the graph labeled 'Blue light 1hr - control'*
1. Aseptically
Pronunciation link: <https://www.merriam-webster.com/dictionary/aseptically>
IPA: /eɪˈsep.tɪ.kli/
Phonetic Spelling: ay·sep·tuhk·lee
 2. Tryptic
Pronunciation link: <https://www.merriam-webster.com/dictionary/tryptic>
IPA: /ˈtrip.tɪk/
Phonetic Spelling: trip·tik
 3. Staphylococcus aureus
Pronunciation link: <https://www.howtopronounce.com/staphylococcus-aureus>
IPA: /ˌstæfɪləˈkɔːkəs ˈɔːriəs/
Phonetic Spelling: staf·uh·luh·kaa·kuhs aw·ree·uhs
 4. Escherichia coli
Pronunciation link: <https://www.howtopronounce.com/escherichia-coli>
IPA: /ˌɛʃəˈrɪkiə ˈkoʊli/
Phonetic Spelling: esh·uh·rik·ee·uh koh·ly

5. Enterobacter aerogenes
Pronunciation link: <https://www.howtopronounce.com/enterobacter-aerogenes>
IPA: /ˌɛntəroʊbækˈtɪr ˌɛroʊˈdʒiːniːz/
Phonetic Spelling: en-tuh-roh-bak-teer air-oh-jee-nee-z
6. Pseudomonas aeruginosa
Pronunciation link: <https://www.howtopronounce.com/pseudomonas-aeruginosa>
IPA: /ˌsuːdəˈmoʊnəs ɛruːdʒɪˈnoʊsə/
Phonetic Spelling: soo-duh-moh-nuhs eh-roo-jih-noh-suh
7. Acinetobacter baumannii
Pronunciation link: <https://www.howtopronounce.com/acinetobacter-baumannii>
IPA: /ˌæsɪniːtoʊˈbæktəː bɔːˈmɑːniːaɪ/
Phonetic Spelling: as-uh-nee-toh-bak-ter baw-mah-nee-eye
8. Klebsiella pneumoniae
Pronunciation link: <https://www.howtopronounce.com/klebsiella-pneumoniae>
IPA: /ˌklebsiˈɛlə nuːˈmoʊniːiː/
Phonetic Spelling: kleb-see-el-uh noo-moh-nee-ee
9. Candida albicans
Pronunciation link: <https://www.howtopronounce.com/candida-albicans>
IPA: /ˈkændɪdə ˈælbɪkænz/
Phonetic Spelling: kan-duh-duh al-bih-kanz
10. Turbidity
Pronunciation link: <https://www.merriam-webster.com/dictionary/turbidity>
IPA: /tɜːˈbɪdəti/
Phonetic Spelling: tur-bid-uh-tee
11. McFarland
Pronunciation link: <https://www.howtopronounce.com/mcfarland>
IPA: /mækˈfɑːrlənd/
Phonetic Spelling: muk-far-luhnd
12. Antimicrobial
Pronunciation link: <https://www.merriam-webster.com/dictionary/antimicrobial>
IPA: /ˌæn.ti.maɪˈkroʊ.bi.əl/
Phonetic Spelling: an-tee-my-kroh-bee-uhl
13. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪkroʊ.liːtə/
Phonetic Spelling: my-kroh-lee-terz
14. Suspension
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>

IPA: /sə'spenʃən/

Phonetic Spelling: suh·spen·shuhn

15. Irradiance

Pronunciation link: <https://www.merriam-webster.com/dictionary/irradiance>

IPA: /ɪ'reɪdiəns/

Phonetic Spelling: ih·ray·dee·uhns

16. Fluence

Pronunciation link: <https://www.howtopronounce.com/fluence>

IPA: /'flu:əns/

Phonetic Spelling: floo·uhns

17. Irradiation

Pronunciation link: <https://www.merriam-webster.com/dictionary/irradiation>

IPA: /ɪ'reɪdi'eɪʃən/

Phonetic Spelling: ih·ray·dee·ay·shuhn

18. Vortex

Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>

IPA: /'vɔ:rˌtɛks/

Phonetic Spelling: vor·teks

19. Sabouraud

Pronunciation link: <https://www.howtopronounce.com/sabouraud>

IPA: /,sæbʊ'rɒʊ/

Phonetic Spelling: sab·uh·roh

20. Nosocomial

Pronunciation link: <https://www.merriam-webster.com/dictionary/nosocomial>

IPA: /,nɔ:sə'kɒsmiəl/

Phonetic Spelling: nah·suh·koh·mee·uhl

21. Nebulizer

Pronunciation link: <https://www.merriam-webster.com/dictionary/nebulizer>

IPA: /'nɛbjələˌzaɪzə/

Phonetic Spelling: neb·yuh·luh·zy·zer

22. Spectroscopy

Pronunciation link: <https://www.merriam-webster.com/dictionary/spectroscopy>

IPA: /spɛk'trɔ:skəpi/

Phonetic Spelling: spek·traa·skuh·pee

23. Collagen

Pronunciation link: <https://www.merriam-webster.com/dictionary/collagen>

IPA: /'kɔ:lədʒən/

Phonetic Spelling: kaa·luh·juhn

