SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA17107
STSM title: Evaluation of the antimicrobial activity of chlorinated polyimide membranes
STSM start and end date: 13/09/2021 to 01/10/2021
Grantee name: Ewa Sroczyk

PURPOSE OF THE STSM:

The purpose of my STSM was to evaluate the antimicrobial activity of the chlorinated polyimide (PI) patches and to establish long-term cooperation between AGH UST and the University of Birmingham.

The main problem I focus on is atopic dermatitis. This chronic disease is characterized by, among others, itchiness, disrupted skin barrier, and relapsing inflammatory. In addition, an increased amount of *S. Aureus* bacteria has already been confirmed by atopic patients. Therefore, it is recommended by dermatologists to take “bleach baths”. The bleach baths are prepared by dilution of sodium hypochlorite and are beneficial for atopic skin. So as to avoid soaking the whole body, I decided to design convenient chlorinated patches (as the active chlorine is the active component of the bleach baths), which would be used on selected parts of the body.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

I electrospun the PI membranes and also chlorinated them at the AGH UST before the STSM beginning. During my STSM, I learned to conduct bacteria culture and perform antimicrobial assays on polymer membranes.

I prepared the Luria broth (LB) agar plates that are used for bacteria growing. LB is a nutrient-rich media, and the addition of agar results in the formation of gel that bacteria can grow on. The LB agar plates were prepared out of yeast extract (0.5 %, Sigma Aldrich), tryptone (peptone from casein, 1 %, BD Diagnostics), NaCl (1 %, Sigma Aldrich), and agar (1.5 %) in water (grade II). This solution was sterilized at 121 °C for 15 min and then poured onto a petri dish in a sterile environment (provided by Bunsen burner). Two bacteria strains (*S. Aureus*, ATCC6538 and *E. Coli,* ATCC25922) were grown in LB and then diluted with PBS. The concentrations of the bacteria solutions were evaluated with UV-VIS spectrophotometer Ultrospec 2100 pro (Biochrom, US) at wavelength 600 nm. Then I recalculated the obtained and desired optical density 0.1 and prepared the desired bacteria solutions. For the tests, I used the LB solution as a control to exclude the contamination of the growing medium.

So as to determine the antibacterial activity of the membranes, I used Dey-Engley neutralizing broth (Sigma Aldrich) in concentration 3.9 %. This broth neutralizes a broad spectrum of antiseptics and disinfectants, including chlorine, and allows for reliable disinfectants testing. The solution was sterilized at 121 °C for 15 min.

I conducted this experiment in two ways according to the different material properties (hydrophobicity and hydrophilicity of the membranes).

1st way (scheme in figure 1): the membranes were put into 15 ml testing tubes. Afterward, I poured the 250 µl of bacteria solutions into proper tubes and let them grow at temp. 22 °C for 16 h. Then I added 10 ml of the neutralizing broth, which stops disinfectants activity. Such solutions were diluted into 8 sequential dilutions, with a 1/3 decrease in concentration. Finally, 10 µl drops of these 8 solutions were cultured again at 37 °C for 16 h onto a new agar plate divided into 8 equal parts (see figure 2).

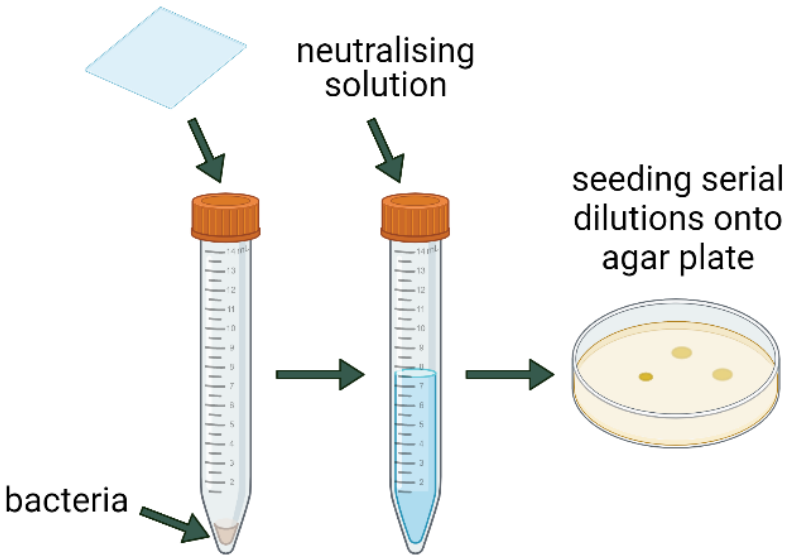

Figure 1. The scheme of the antimicrobial test – putting membranes into tubes (1st way).

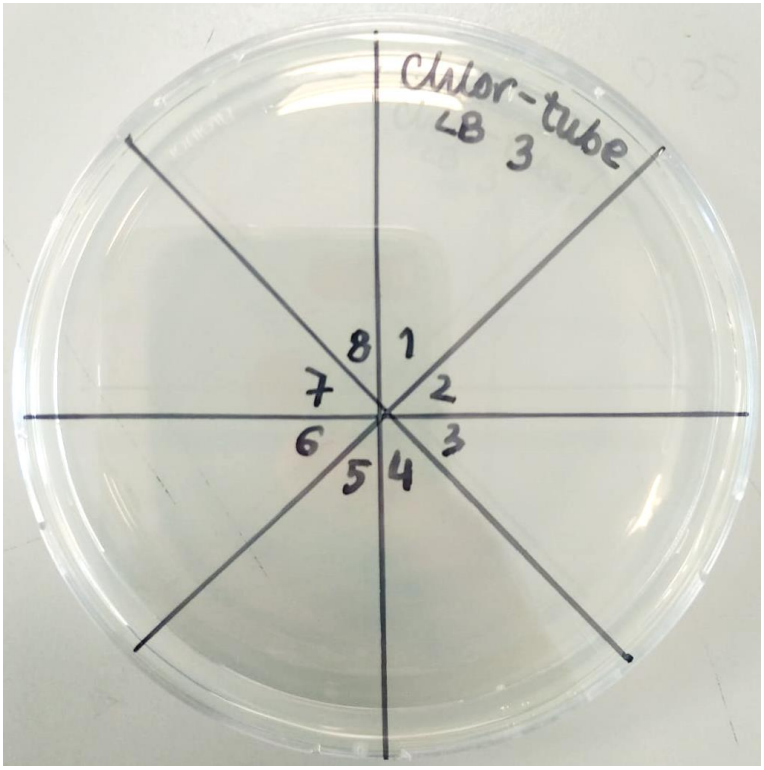

Figure 2. The representative division of the agar plates for seeding serial dilutions.

2nd way: the membranes were put onto a 24-well plate. Afterward, I put the 40 µl droplet of bacteria solutions onto the membranes' surfaces and let them grow overnight. Then I added 10 ml of the neutralizing broth and conducted the experiment in the same manner as way 1 – see figure 3.

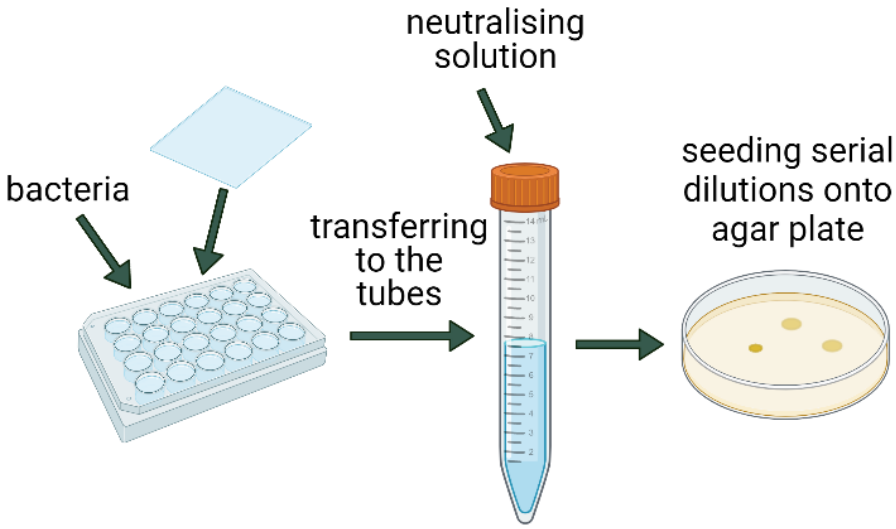

Figure 3. The scheme of the antimicrobial test – putting membranes in the 24-well plate (2nd way).

The final agar plates were imaged with Bio-Rad Molecular Imager ChemiDoc XRS+ with ImageLab v. 6.0 software (Bio-Rad Laboratories, Inc). Then, the countable dilutions were selected, where the colony-forming units (CFU) of bacteria were less than 50 in one droplet, counted, and compared within the replicates.

I thoroughly discussed the results with the supervisor both during and also after the experiments.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

I assessed the pictures for S. Aureus and E. Coli for the amount of CFUs. The example picture of the 8 sequential dilutions of bacteria after 16 h of growth is shown in figure 4.

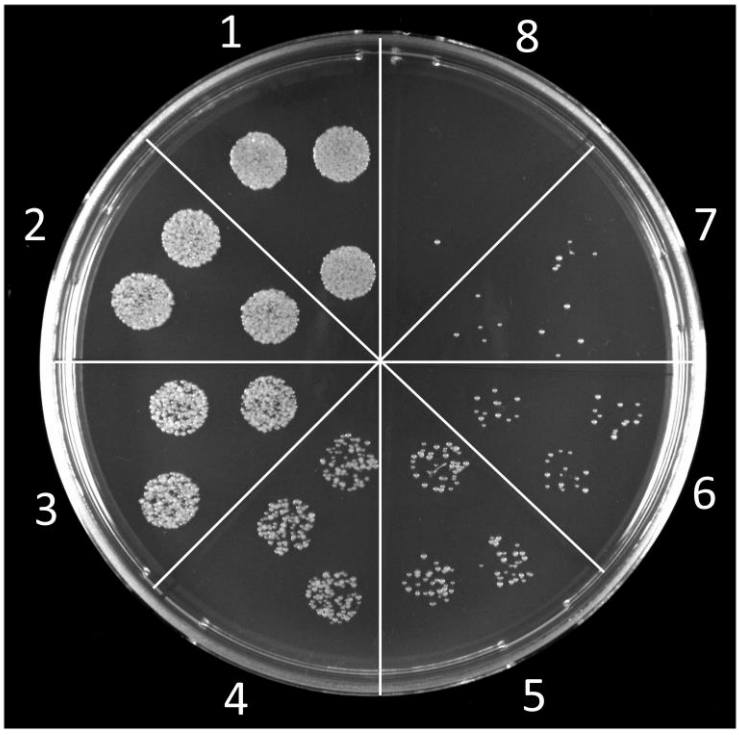

Figure 4. The agar plate with sequential dilutions of *S. Aureus* grown on the PI membrane (2nd way).

Based on such pictures, I recalculated the CFUs with proper dilution concentration and prepared the graphs for both methods- see figure 5.

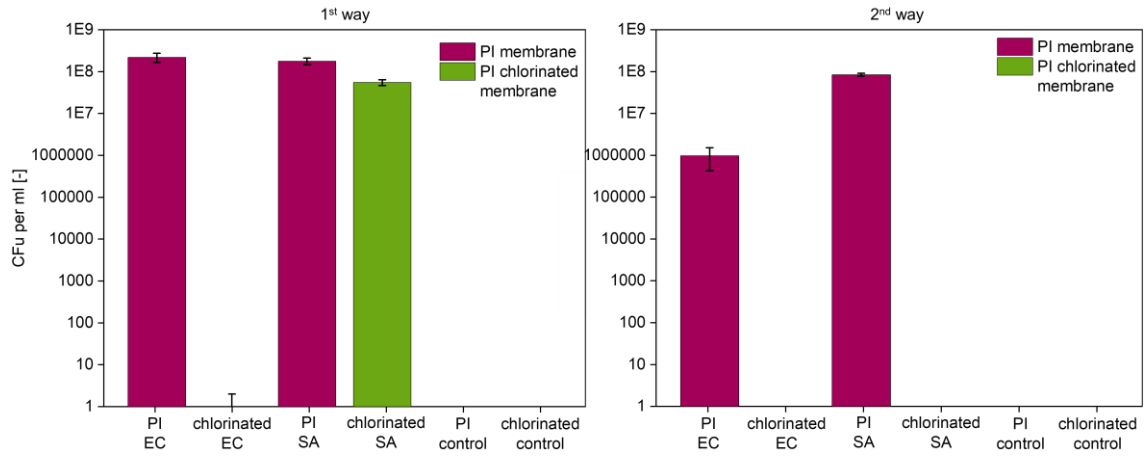

Figure 5. Comparison of the antimicrobial activity for PI and chlorinated membranes, for *S. Aureus* (SA), *E. Coli* (EC), and LB (control).

The results clearly indicate the antimicrobial activity of the chlorinated PI membranes. Both bacteria strains are present on the PI membranes. LB used as a control to exclude the contamination does not exhibit any bacteria, so the experiments are reliable.

For the 1st method, the *S. Aures* are present both on the PI and chlorinated membranes, suggesting the more potent antimicrobial activity for gram-negative bacteria- *E. Coli*. For the 2nd method, both bacteria strains are only on the PI membranes, which means the antimicrobial activity comes directly from the chlorinated membranes, not from the material architecture nor other material properties.

These experiments conclude that the chlorinated PI patches have antimicrobial properties, and the active chlorine concentration on the membranes is high enough to inhibit bacteria growth. The obtained results have already been discussed with the supervisor and compared with the literature. We will use them in the currently prepared manuscript. Our outcomes bring a great promise for future research and possible application for skin patches, not only for atopic skin treatment but also for other skin infections healing.

FUTURE COLLABORATIONS (if applicable)

These research methods will be used to assess the antimicrobial activity of other polymer membranes with the Host institution. The results obtained during this STSM will be published in a scientific paper.