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 antibacterial properties of electrospun fibers enriched with TiO_2 particles
STSM start and end date: 25/04/2022 to 13/05/2022
Grantee name: Joanna Karbowniczek

PURPOSE OF THE STSM:

The main purpose of the STSM was to evaluate the antibacterial properties of electrospun PHBV fibers with added titanium dioxide (TiO_2) nanoparticles. Such fibrous materials are intended to serve as scaffolds in tissue engineering applications. While creating a beneficial microenvironment for cells attachment and tissue development also bacterial growth might be promoted. Based on literature review TiO_2 after photoactivation with UV present some antimicrobial activity, however it is strongly related with the content, form and distribution of nanoparticles. In this work two approaches based on electrospinning process were proposed to prepare composite organic-inorganic fibers. Namely blend electrospinning with clusters of nanoparticles partially embedded in polymer matrix and application of core-shell needle were PHBV fibers are decorated with TiO_2 nanoparticles. Microbiological testing was essential to evaluate the antibacterial activity of prepared materials.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Frist part of the work was samples preparation, which was done at AGH University of Science and Technology in Krakow. I manufactured 3 types of electrospun materials: **PHBV** fibers (only polymer materials to be used as reference sample); **blend** samples obtained from mixed solution of PHBV polymer and TiO_2 nanoparticles; core-shell (**C-S**) fibers where core is PHBV and outer shell is composed of nanoparticles. Additionally, scanning electron microscopy (SEM) imaging was done to confirm the structure and composition of materials. During the STMS at University of Birmingham the antibacterial tests were performed. Samples with and without UV irradiation (for 30 min) were tested against Gram- bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram+ *Saphylococcus aureus* with standard surface testing protocol. Also disc diffusion test was done to verify if materials are leaching any antibacterial agents. Finally, live/dead staining and confocal microscopy imaging was performed for samples after UV exposure for 30 min and 18 hours of *E. coli* incubation. Another set of samples after UV exposure for 30 min and 18 hours of *E. coli* incubation were fixed and dehydrated for SEM imaging, to be done AGH-UST in Krakow.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

By SEM imaging of as received electrospun materials significant morphological differences between 3 samples were observed, see Fig.1. PHBV fibers had smooth morphology and average fiber diameter of about 3.2 µm. For both hybrid samples fiber diameter increased, till 3.9 µm for blend sample and 3.5 µm for

C-S. Additionally, in blend sample multiple particles aggregates were observed causing beads-like structures to be formed along the fibers. While C-S fibers had wrinkled surface with homogenously distributed TiO_2 nanoparticles.

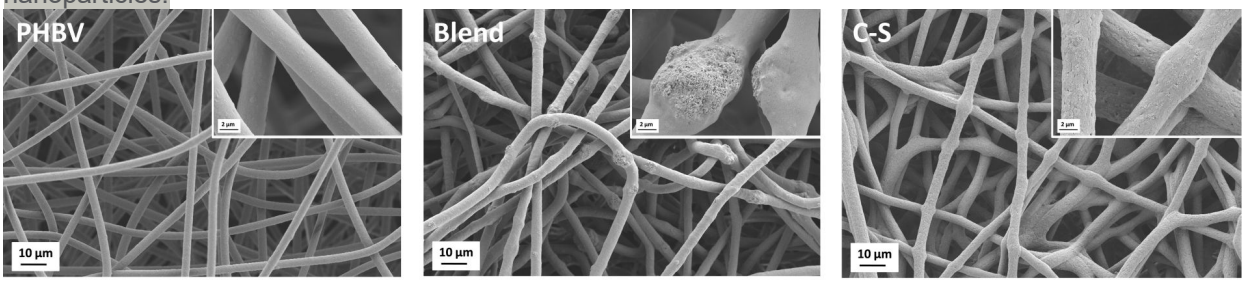

Fig. 1. SEM micrographs showing the morphology of as received electrospun fibers.

Initial antibacterial test for surfaces not exposed to UV showed no reduction in colony forming units (CFU)/ml when tested with S. aureus and some reduction with E. coli. Furthermore, there was no inhibition of bacterial growth around the materials in disc diffusion test, suggesting no leaching of antibacterial agents from samples. Tests performed on UV exposed samples showed no effect on S. aureus and significant reduction of CFU/ml with E. coli , as shown in Fig. 2a (10^9 CFU/ml in no material control and around 10^4 CFU/ml for electrospun samples). Testing with another Gram- bacteria - P. aeruginosa showed some reduction in CFU/ml due to contact with electrospun samples, but not that evident as for E. coli. Live/dead staining and confocal imaging allowed to confirm presence of multiple dead E. coli bacteria on C-S sample (Fig. 2b), for other materials results were not that clear. We should receive additional information based on SEM imaging of materials with fixed bacteria.

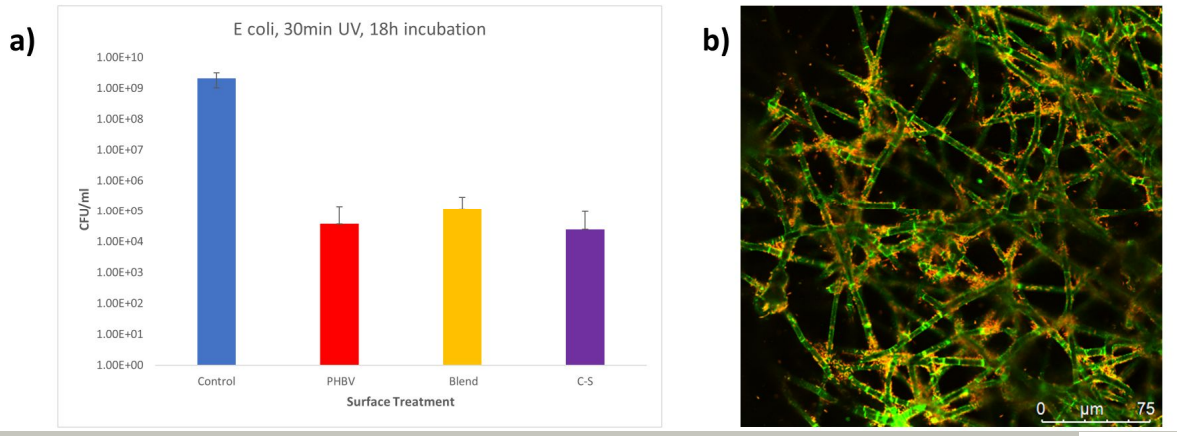

Fig. 2 a) Number of CFU/ml for test with E.coli incubated for 18 hours on surface o UV activatedf electropun matrials, b) confocal image of live.dead staingn on C-S sample (red indicating dead bacteria cells).

The STSM at University of Birmingham gave me an excellent opportunity to work with Dr Felicity de Cogan and her team. I could gain new knowledge and experience in terms of antibacterial studies. Within 3 weeks of my stay in UK we could perform multiple tests and receive interesting data that after detailed analysis and interpretation will be presented in joint publication.

FUTURE COLLABORATIONS (if applicable)

Based on results obtained within this STSM a joint publication will be prepared.
There are perspectives of further collaboration regarding antibacterial testing of other electrospun materials.