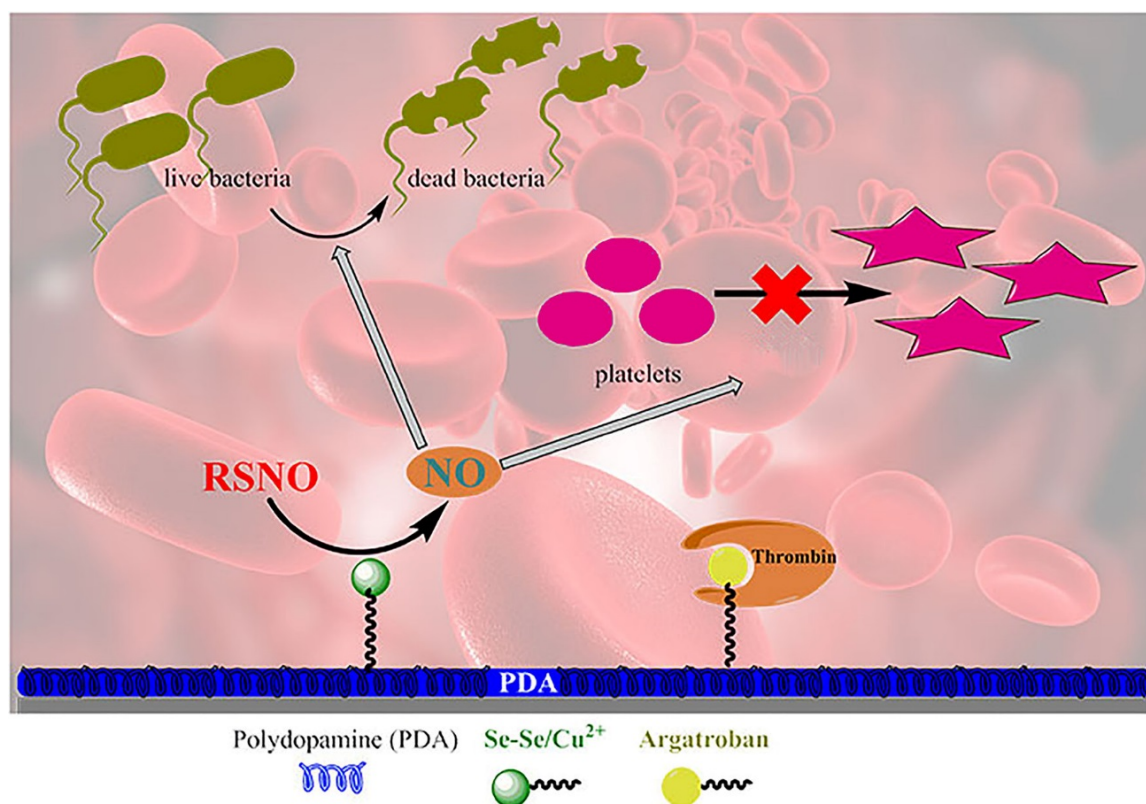


2017- 2019 DUALFUN: Development of blood-contacting materials with dual antithrombotic and antibacterial function. Horizon 2020 Marie Skłodowska-Curie Actions (MSCA) Individual Fellow grant.



Surface-induced thrombosis and infection are two main complications which cause failure of medical devices. Device-related thrombosis is associated with activation of blood clotting and platelets adhesion and activation. Infection of the implanted devices occurred as a result of bacteria adhesion to the biomaterial surface. Essentially biocompatible polymer for blood contacting devices should have both antithrombotic and antibacterial functionalities as both types of complication lead to an increase in morbidity, extended hospital stay and mortality.

In order to improve the resistance of blood contacting materials to both, thrombosis and infection, a novel approach that includes construction of multifunctional coatings, with two biological functions: generation of nitric oxide (NO) and direct inhibition of thrombin by the surface-bound thrombin inhibitor Argatroban was undertaken.

Two the most commonly used synthetic polymers; polyurethane (PU) and polyvinylchloride (PVC) were modified in term to obtain the materials with dual function. The chemical and physicochemical analysis of obtained polymers confirmed that desired modifications were obtained.

Biological testing of modified polymers confirmed generation of NO by both polymers in the range of physiological level. Antithrombotic analysis of the obtained materials showed high effectiveness and sensitivity towards thrombin inhibition and ability to inhibit platelet adhesion. Both modified polymers displayed ability to prevent bacterial adhesion. As a conclusion, the novel polymer materials with dual function – thromboresistant and antimicrobial – were produced.

The project was hosted by the multi-disciplinary Biomaterials and Medical Devices Research group at School of Pharmacy and Biomolecular Sciences, University of Brighton. This project is a continuation of the research from the previous FP6 IRSES Compositum and FP7 IRSES Nanobiomat projects, completed by the group, in which Dr Liana Azizova took part as a visiting Research Fellow.

The DualFun project has received funding from the Marie Skłodowska-Curie Actions (MSCA) Individual Fellows (IF) under the European Union's Horizon 2020 research and innovation programme under grant agreement number 749207.

Project aims

The key aim of this project is to develop novel dual function polymer materials to prevent device-induced thrombosis and infection.

This aim will be achieved by:

- chemical attachment of the organoselenium or copper nanoparticle catalysts to the polymer surface in order to continuously generate NO by decomposition of endogenous S-nitrosothiols
- immobilisation of the direct thrombin inhibitor argatroban to inhibit any thrombin in the surrounding environment.

Catecholamines, polydopamine and poly(norepinephrine) will be used as the surface modification reagents, as they form very stable thin films with reactive ligand strongly attached to the polymer surface. Owing to the chemical bonding of the ligand to the polymer surface it is expected that these materials will have long storage life and exploitation period and therefore retain their ability to generate NO from the inexhaustible endogenous NO donors and prolong activity of bound thrombin inhibitor.

Project findings and impact

Research team

Dr Liana Azizova, Marie Skłodowska-Curie Actions (MSCA) Individual Fellow

Dr Lyuba Mikhalovska

Dr Jim Cunningham

Dr Iain Allan

I. Project results

PVC and PU polymers modified with Cu and Se for catalytic NO generation were produced. Further thrombin direct inhibitor Argatroban was chemically attached onto the Cu/Se-modified polymers surface. All obtained materials were characterized by physico-chemical methods such as ICP-OES, ICP-MS, XPS, FTIR. The NO generation ability of polymers in both PBS and human plasma were tested and showed that Argatroban Cu/Se-modified polymers are capable of providing the physiological level of NO at physiological pH. The obtained data suggest that argatroban Cu/Se-modified polymers potentially can produce nitric oxide in the blood from endogenous S-nitrosothiols. Additionally, Argatroban Cu/Se-modified PVC and PU effectively inhibited thrombin activity in buffer system and in platelet poor plasma or platelet rich plasma, thus preventing the platelet activation and aggregation. Another important achievement is that Argatroban Cu/Se-modified polymers showed antibacterial effect during 2h of incubation with *S. aureus* and *E. coli* in the presence of 100 μ M GSNO/GSH in PBS (Fig. 1).

Cu-coated polymers by magnetron-sputtering technique were obtained in Teer Coatings Ltd in collaboration with Dr Parnia Navabpour and Dr Hailin Sun and tested for NO generation ability.

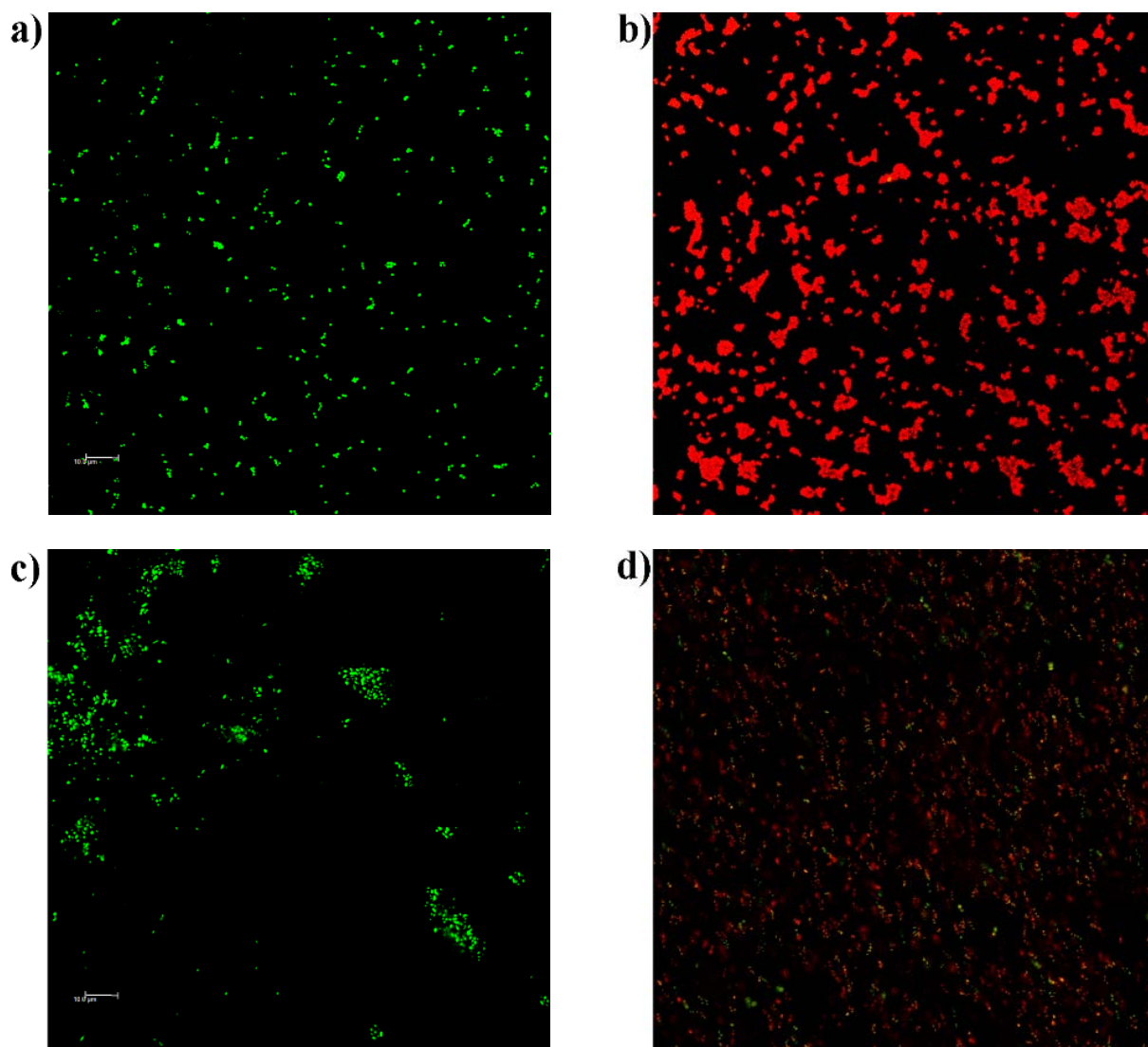


Fig. 1. *S. aureus* and *E. coli* biofilm with Live/Dead staining and imaged with LSCM. The green signal indicating alive cells. The red signal marks the dead cells. a) control

of living biofilm *S. aureus* on PVC; b) biofilm *S. aureus* on PVC/Cu/AG after 2hrs of incubation of bacterial inoculum with polymer and 100 μ M GSNO/GSH; c) control of living biofilm *E. coli* on PVC; d) biofilm *E. coli* on PVC/Cu/AG after 2hrs of incubation of bacterial inoculum with polymer and 100 μ M GSNO/GSH.

II. Secondments

1. Teer Coatings Ltd, Miba Coating Group (Dr. Parnia Navabpour, Dr. Hailin Sun)

Presentation in Teer Coatings, 31 July 2018



Presentation in Teer Coatings Ltd. It was productive meeting with rewarding discussions



Achievements:

In collaboration with Dr Parnia Navabpour and Dr. Hailin Sun (Teer Coatings Ltd. UK) Cu coated PVC and PU by unbalanced magnetron sputtering technique have been obtained.

I was trained in a number of techniques for the investigation of mechanical properties of coatings, such as wear-resistance, scratch resistance and hardness. This included:

- pin on disc tester
- scratch and wear tester
- ball crater equipment
- abrasion tester
- hardness tester, both Rockwell and Vickers

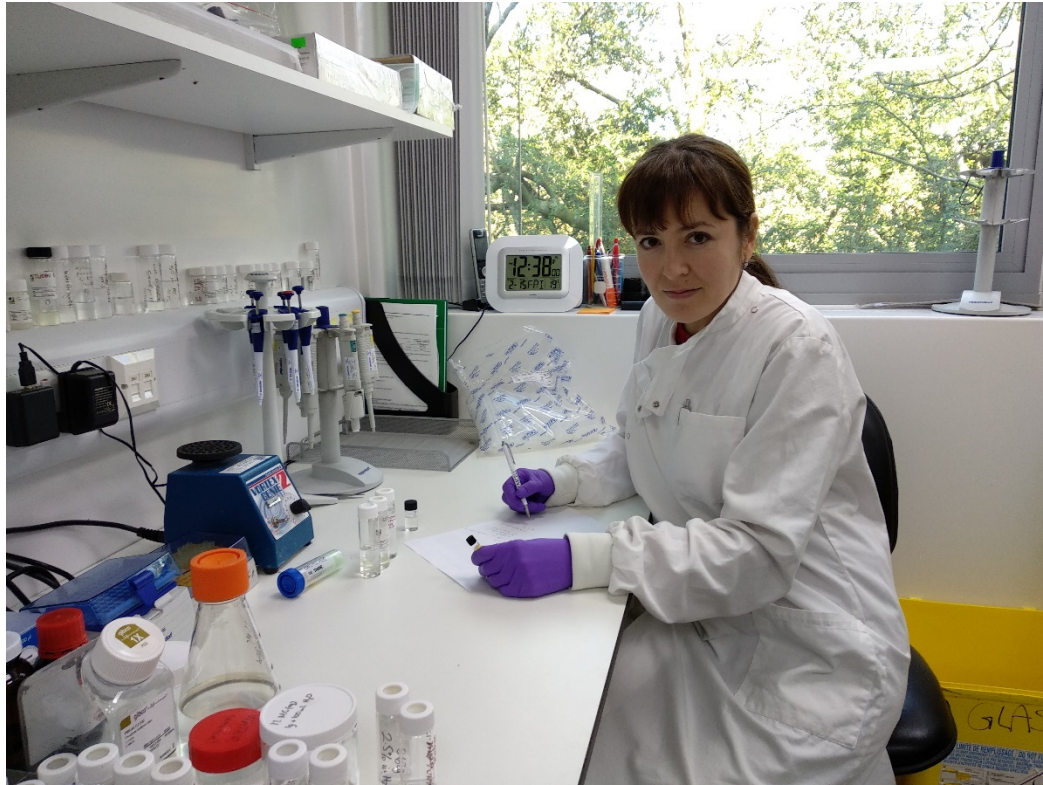
2. Pharmidex Ltd (Prof. Mo Alavijeh, Dr Anil Misra, Dr Rostislav Shevchenko)

Achievements:

I made a presentation of my current research in Pharmidex, Hatfield, UK. 31 January 2019. During secondment I gained knowledge in new methods in clinical studies, drug discovery, gained experience in some clinical trials and tested my knowledge in mass spectrometry trained in the main requirements of GLP in research laboratories in pharmacy.

- trained and performed the assay on validation of biomaterials toxicity (MTT assay).

- obtained supervision for performing the hemolysis assay
- gain knowledge on new methods in drug discovery and drug development
- establish collaboration with Dr Mohamed Alavijeh, Dr. Anil Misra and Dr Rostislav Shevchenko



III. Conferences

1. **ISBPPB 2018** - 4th International Conference on Biomedical Polymers & Polymeric Biomaterials, July 15-18, 2018, Krakow, Poland.





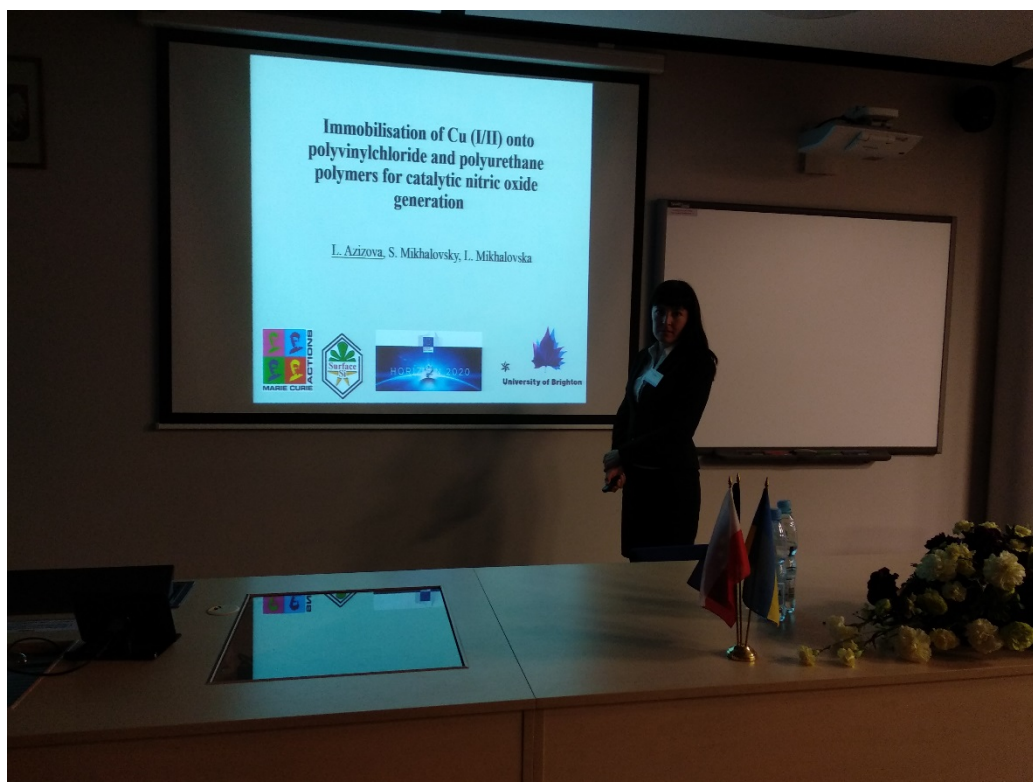
The 4th International Society for Biomedical Polymers and Polymeric Biomaterials conference and exposition is being jointly organized by the International Society for Biomedical Polymers and Polymeric Biomaterials, Polish Society for Biomaterials, and Faculty of Materials Science and Ceramics of the AGH University of Science and Technology. First time the ISBPPB conference was taking place outside the United States. Around 300 participants attended the conference from Austria, Belgium, Belarus, Czech Republic, Finland, France, Germany, Greece, Italy, UK, the Netherlands, Romania, Russia, Spain, Switzerland, Mexico, Australia, South Africa, Brazil, India, Korea, Turkey and USA. The Conference covered topics such as surface modification and functionalization, advanced manufacturing, biosensors, encapsulation and controlled release, drug delivery systems, cardiovascular applications, antimicrobial polymers, biodegradable polymers and copolymers, tissue engineering, neural regeneration, wound healing/soft tissue engineering and many others.

My presentation got attention of the representative of Arburg company, one of the leading global manufacturers of plastic processing machines, which was one of sponsors of the conference. I have got certificate of attendance of conference.

2. 16th Polish — Ukrainian Symposium on Theoretical and Experimental Studies of Interfacial Phenomena and their Technological Applications, Lublin, Poland, August 28-31, 2018

196 reports on theoretical and experimental studies of interfacial phenomena, in particular, adsorption and gas chromatography, catalysis, ion exchange and problems related to human health care and environmental protection were presented on Symposium by researchers from academia and industry. These studies are summarised in the Symposium Abstracts volume.

My oral presentation was interesting to audience and evoke fruitful discussions.



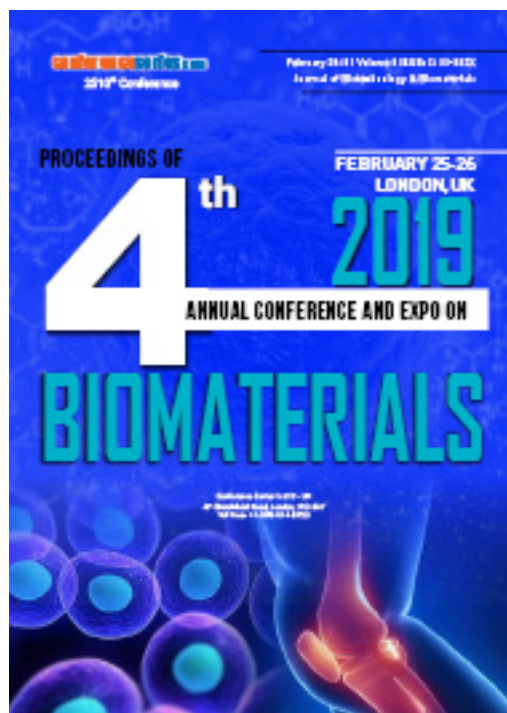
3. The 29th Annual Meeting of the European Society for Biomaterials, September 9–13, 2018, Maastricht, the Netherlands.

The 29th ESB 2018 I attended covered various topics in the field of biomaterials. This conference also acted as a platform to learn new ideas and findings in the field of biomaterials. Numerous national and international top scientists give a plenary lecture and covered many interesting topics the field of biomaterials. There were a lot of very interesting lections dedicated to new technologies in orthopaedics, ophthalmology, cardiovascular and neurodegenerative diseases from scientists, clinicians and industrialists with different backgrounds and expertise.



Has become a member of the European Society for Biomaterials in 2017.

4. 4th Annual Conference and Expo on Biomaterials, February 25-26, London, UK



The conference was attended by young researchers, students and business delegates. This year the theme of the conference is “Exploring the advancements of biomaterials in engineering and health sciences”. The conference reports were printed in Proceedings of 4th Annual Conference and Expo On Biomaterials 2019: Journal of Biotechnology and Biomaterials, Volume 9

IV. STEM and outreach activity

1. STEM activity at Big Bang South East 2018, 27 June 2018, Ardingly, UK
(https://www.youtube.com/watch?v=QJNHJA_BQtM).

It is organised by STEM Sussex, the outreach department of the University of Brighton in order to inspire young people to study and pursue science, technology, engineering and maths. More than ten thousand young people from 250 schools have attended Big Bang South East 2018.



2. STEM activity at Big Bang Brighton 2018, 11 July 2018, Brighton, UK.

The STEM activity at the University of Brighton (Brighton) organised by STEM Sussex, the outreach department of the University of Brighton in order to encourage more students to take up STEM subjects and careers and discover available future opportunities.



Activities

UKSEDS

Space for learning

UK Students for the Exploration and Development of Space is the UK's national student space society with the mission of supporting students and enthusiasts through outreach, running space projects and hosting conferences.



University of Brighton

Stop the Clot

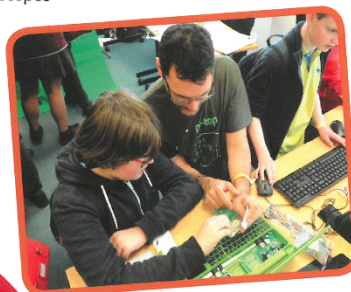
Visit the **University of Brighton** School of Pharmacy and Biomolecular Sciences to learn about blood composition and the function of different blood cells in the body. Look through microscopes at blood smears to see how blood cells really look close up. Find out about platelets, atherosclerosis plaque development, stents and other medical devices to treat cardiovascular diseases.



WWT Arundel Wetland Centre

STEM in nature

Drop in session – come along to our stand and meet WWT Arundel's learning team. They will be on hand to talk to you about the various jobs needed to run a successful nature reserve, from the outdoor teachers who deliver learning sessions for visiting schools to the grounds team who look after the reserve and its wildlife. We will demonstrate some of our survey techniques used to monitor the wildlife on the reserve and allow you opportunity to get hands on with the artefacts we use in our learning sessions.



12

3. STEM activity at Big Bang South East 2019, 27 June 2019, Ardingly, UK.

(https://www.stemsussex.co.uk/wp-content/uploads/2019/08/STEM_SUSSEX_2019_H264-1.mp4?_t=1)

I took part on 8th Big Bang Fair South East organised by STEM Sussex, the STEM outreach department at the University of Brighton. More than 13000 young people between the ages of nine and 19 and their teachers attended and took part in festival activities.

V. Activity at the Brighton University

Lectures and presentations

1. Lecture to master students, at University of Brighton, 3.05.2018. (CHM01 module)
2. Lecture to master students, at University of Brighton, 21.11.2018.
3. Lecture to master students, at University of Brighton, 11.02.2019. (CH327 module)
4. Training and lecture for students about NO release detection using by $\text{NO}_2^-/\text{NO}_3^-$ ion selective electrodes, 5.02.2019.
5. Seminar at Biomaterial and medical device group, PABS, 18.12.2017.
6. Seminar at Biomaterial and medical device group, PABS, 31.07.2017
7. Seminar at Biomaterial and medical device group, PABS, August 2019

Training and other activities

I supervised 2 students and co-supervised 3 undergraduate students. I planned project and research for students from PABS on a daily basis. This helped me to increase the strength of my supervision skills and improved my leadership, time management abilities, delegation of responsibilities.

The list of supervised students:

1. Lydia Smith - September 2017 – May 2018. Encouraged by research project Lydia Smith applied for PhD programme at the University of Nottingham.
2. Simon Jay - September 2017 – May 2018.
3. Toral Patel - December – April 2019
4. Sophia Khan - December – April 2019
5. Edward Plugge - December – April 2019

List of trainings:

During the fellowship I had various trainings to gain new skills.

1. University Induction Day UoB, 13th of October 2017.
2. the Equality and Diversity Essentials training, 14 November 2017
3. Research and human tissue legislation, 19 January 2018
4. HTA steering group meetings (had role of research representative the School's HTA Steering Group), 6.03.2018 and 14.03.2019.
5. Training in Flow cytometry by Dr Yishan Zheng and Anna Blunden (Senior Technician Biological Sciences), 2.05.2018
6. Training in platelet adhesion assay by Dr Lyuba Mikhalovska, 5.05.2018
7. Pedagogic Research Conference, University of Brighton, 1 February 2019
8. Introduction to GCP for IMP Management (Pharmacy), 3 May 2019
9. The theory and operations of the QExactive Orbitrap mass spectrometer by Dr. Andrew Williamson from Thermo Fisher, 5.06.19
10. "Introduction to Good Clinical Practice (GCP)" eLearning, 18 July 2019
11. Training in confocal laser scanning microscopy by Dr. Iain Allan, 07.2019
12. Training in Hemolysis test by Dr. Anil Misra (Pharmidex), 2.03.2019
13. Training in MTT assay by Dr. Anil Misra (Pharmidex), 17.05.2019
14. Gram staining training by Dr Iain Allan
15. Prince2 Agile training
16. Agile PM training

Acknowledgements

I would like to express my sincere gratitude to School of Pharmacy and Biomolecular Science, University of Brighton, for giving me such opportunity to work in such project. This work is supported by the Marie Skłodowska-Curie Action of the European Union (H2020- MSCA-IF-2016, grant agreement no. 749207).

Also, I want to thank to Dr. Lyuba Mikhalovska, Prof. Sergey Mikhalovsky, Dr Jim Cunningham, Prof. Mo Alavijeh, Dr Anil Misra, Dr Rostislav Shevchenko, Dr Parnia Navabpour, Dr Hailin Sun, Dr. Susan Sandeman, Dr Thomas Modine for their help and for the opportunities I was given to conduct my research. My acknowledgement goes to all the technicians of School of Pharmacy and Biomolecular Science and School of

Environment and Technology, University of Brighton for their co-operations. Also, I want to express my gratitude to school office and research office staffs for their support.