**PROGRESS REPORT RISE I**

**1. General Progression of the action**

**1.1 Please indicate the progress of the action during the period covered by this report:**

The action has fully achieved its objectives for the period.

The action has achieved most of its objectives for the period with relatively minor deviations.

The action has achieved some of its objectives but corrective action is required.

The action has failed to achieve critical objectives and/or is severely delayed.

**The action has achieved most of its objectives for the period with relatively minor deviations**

**1.2. General scientific progress of the action**

**The planned objectives of the PANG project are**

**Objective I:** Development of graphene-based antibacterial matrixes though chemical functionalization of these structures using antibacterial peptides and molecules (e.g. menthol and others) and test them for their toxicity and bactericidal potential (in particular against AMR strains)

**Objective II:** Use of the novel architectures in form of suspensions and transdermal patches for the killing of pathogens *via* non invasive photothermal therapy taking advantage of the good photothermal properties of rGO

**Objective III:** To get a deeper understanding of the effects of the novel structures on the immune system

**Objective IV:** To develop prototypes of antibacterial graphene matrixes and antibacterial transdermal patches with the SMEs involved in the project

The first phase of the project was dedicated to the **first two objectives of PANG** notable developing different graphene-based antibacterial matrixes and testing them for their bacteriocidal potential. The use of non invasive photothermal therapy taking advantage of the good photothermal properties of the graphene nanostructures was developed in details during this periode in addition.

This part of the work was motivated by the fact, that despite the availability of different antibiotics; bacterial infections are still one of the leading causes of hospitalization and mortality. The clinical failure of antibiotic treatment is due to a general poor antibiotic penetration to bacterial infection sites as well as the development of antibiotic resistant pathogens. Alternative approaches for the treatment of bacterial infections are crucially needed.

We developed a flexible skin patch (**Figure 1**) allowing a rapid and highly efficient treatment of subcutaneous wound infections *via* photothermal irradiation (**objectives I, II)** The skin patch combines the near infrared photothermal properties of a gold nanohole array (Au NHs) formed by self-assembly of colloidal structures on flexible polyimide films with that of reduced graphene oxide (rGO) nanosheets for laser-gated pathogen inactivation. *In vivo* tests performed on mice with subcutaneous skin infection and treated with the photothermal skin patch showed wound healing of the infected site, while non treated areas resulted in necrotic muscular fibers and bacterial infiltrate. No loss in efficiency is observed upon multiple uses of these patches due to their robustness. Our approach enables the treatment of skin infections in an antibiotic-free manner. This paper is currently under consideration in Small. We are currently developing this approach further by including different antibiotics for in vivo tests in mice with skin infections.

1. **(B)**

***Figure 1.*** *(A) Developed flexible nanoholey patches for antibiotic free treatments of skin infections: (B) Schematic illustration of the fabrication of a flexible photoactive skin-patch based on ODS-loaded rGO deposited onto Kapton for transdermal drug delivery ( Journal of Controlled Release, 2017, 245, 137-146*

In parallel to this, and in line with the strong focus was on demonstrating the concept of using photothermal heating patches for transdermal drug delivery (**objective II**), a skin-mounted patch capable of controlled transcutaneous delivery of therapeutics was developed. Due to initial difficulties using antibiotics as drugs, we used ondansetron (ODS), a commonly used drug for the treatment of chemotherapy-induced nausea and vomiting as model compound. It could be shown that ODS loaded Kapton/rGO patches have a high drug delivery performance upon irradiation with a continuous laser beam at 980 nm for 10 min due to an induced photothermal heating effect of the grpahene loaded patch (**Figure 2**). The ability of ODS impregnated Kapton/rGO patches as transdermal delivery scaffolds for ODS across the skin is in addition investigated using porcin ear skin as a model. We showed that the cumulative quantity and flux of ODS passing the skin are highly depending on the laser power density used. At 5 W cm-2 irradiation, the ODS flux across pig skin was determined to be 1.6 μg cm-2 h-1. The use of tween 20 as skin enhancer could significantly increase the ODS flux to 13.2 μg cm-2 h-1. While the skin penetration enhancement is comparable to that obtained using other well-known permeation enhancers, the actual superiority and interest of the proposed approach is that the Kapton/rGO photoactivatable skin patch can be loaded with a any drugs and therapeutics of interest, making the approach extremely versatile.

To get a deeper understanding of the effects of the novel structures on the immune system (**Objective III**) preliminary studies are under way. It was however already shown (Frontiers in Immunology (section: Molecular Innate Immunity) 2016, 7, 557; Proc Natl Acad Sci U S A. 2016 Oct 4;113(40):E5856-E5865) that carbon nanostructures below 10 nm show different immunogenic responses than larger nanostructures. While these studies were focused on diamond nanostructures, parallel investigations on graphene nanosheets are under development. The entrapment of graphene nanostructures via the formation of neutrophile extracellular traps (NETs) is under investigation.

**Objective IV,** development of prototypes of antibacterial graphene matrixes and antibacterial transdermal patches with the SMEs can only be realistic be achieved at a later stage, as some of the work done in this direction has to be fist consolidated. However, the development of methods for large scale patch preparation using spraying techniques is under way and directly linked to these objects.

**In conclusion**, the successes of the first years can be summarized as following:

1. Construction of a graphene-based flexible skin patch allowing the photothermal treatment of wound infections. Proof of principle by in *vivo* studies on mice with skin infections
2. Use of flexible photothermal heating patches for transdermal drug delivery. Proof of principle by *in vitro* studies using ondansetron and insulin.The on-demand delivery of drugs upon local laser irradiation and the possibility to reload the patches with the drug makes this approach a new drug administration rout. We are currently moving towards integrating antibiotics into this system to see if a similar concept can be applied.
3. We revealed the ability of living organisms to entrap nanostructures *via* the formation of neutrophile extracellular traps (NETs). The cellular mechanism how small nanostructures activate NETosis and drive their entrapping and resolution of the initial inflammatory response is given.

**1.3. Deliverables**

Since the official start of the project January 1sd 2107, the PANG project has fulfilled following intended deliverables (**Table 1**).

**Table 1: Deliverables**

|  |  |  |  |
| --- | --- | --- | --- |
| **Nr.** | **Deliverable name** | **month** | **comments** |
| **WP 1** |
| D1.1 | Project start up | M0 | Kick-off meeting London |
| D1.2. | Consortium agreement | M3 | Done |
| D1.3 | Progress Report | M6 | Finished M9 |
| D1.4. | Progress Report | M12 | M15 |
| **WP 2** |
| D2.1. | Protocol of the synthesis of various ligands for modulation glycan-protein interacts | M18 | Done (Chem NanoMat, 2006; Biology, 2016) |
| **WP 3** |
| D3.1 | Results on bactericidal effects of G interfaces | M24 | paper submitted Small |
| **WP 4** |  |  |  |
| D4.1. | Protocol for the formulation of chemo-photothermal rGO matrixes | M12 | Done (J. Cont. Release 217) |
| D4.2. | Protocol for the formulation of photothermal-photodynamic rGO matrixes | M24 | Done, send delivery protocol |
| D 4.4. | Protocol for the formulation of photothermal rGO patches | M24 | Done (J. Cont. Release 217-2 papers) |
| **WP 5** |  |  |  |
| D5.1.  | PANG website | M3 | Delivered M15 [**http://pang.univ-lille.fr**](http://pang.univ-lille.fr/) |
| D 5.2.  | PANG Newsletter 1 | M12 | Done and uploaded  |

**2. Corrective Measures for secondments**

**2.1. Explain any delays accumulated and measure taken to oversee them**

PANG is behind in the undertaking of secondments (**Table 2**). This is partially based to some initial “starting” problems and getting to know how the exchanges work. However, it was and is mainly due to the current political situation in Turkey and some of the partners are restricted by their institutions formally to make any of the planned secondments there.

**Table 2: Secondements 2016**

|  |  |  |
| --- | --- | --- |
| **Participant** | **2016** | **Total Second.** |
| **USTL** | 9.7 | **24** |
| **RUB** | 0 | **16** |
| **FAU** | 0.6 | **10** |
| **UGOT** | 0 | **5** |
| **LNMU** | 2 | **17** |
| **Graphenea** | 0 | **11** |
| **LSO Medical** | 0 | **11** |
| **RS Research** | 6 | **35** |
| TOTAL | 18.3 | **129** |

**In particular LOS-Medical** has major problems in performing the planned secondements. The reasons are multiple. First, to perform some of the photothermal heating experiments, their 1470 nm laser, unique to them, is needed. As there is currently no provider in the Ukraine, this would mean shipping the instrumentation there. For practical reasons, another scenario would thus be of advantage: Partner LNMU performing the experiments at LSO Medical together with the help of USTL, who is currently dedicating a room at their research institute for *in vitro* photothermal experiments, might be a reasonable solution.

The other issue concerns **Graphenea**. At the current stage of the project, the development of an easy process for the deposition of antibacterial graphene films would be of high importance. Graphene processes a spraying technique. This part of the work should be done here. The USTL team is the most adapted to perform the experiments there and a redistribution of their secondements to USTL would be very beneficial.

A reasonable solution could be the redistribution of these secondments as suggested in **Table 3** without making less secondments as the scientific part of the PANG project advances in line with the planned tasks and has already resulted in not only highly interesting results but also 8 publications, including one cover page and one technical report (see later).

**Table 3: Measures taken to overcome problems with secondments (more details in updated Gantt Tabe)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Participant** | **2016** | **2017** | **2018** | **2019** | **Total Second.** |
| **USTL** | 9.7 | 10 | 10 | 5.3 | **35**  |
| **RUB** | 0 | 6 | 8 | 2 | **16**  |
| **FAU** | 0.6 | 4.4 | 3 | 2 | **10** |
| **UGOT** | 0 | 2 | 2 | 1 | **5**  |
| **LNMU** | 2 | 6 | 7 | 6 | **21**  |
| **Graphenea** | 0 | 2 | 2 | 1 | **5**  |
| **LSO Medical** | 0 | 0 | 1 | 1 | **2**  |
| **RS Research** | 6 | 8 | 10 | 11 | **35** |
|  | **18.3** |  |  |  | **129** |

**2.2. Risks**

WP 3 and 4 are highly advanced and no risk is foreseen. WP1, Management, as well as WP 5 Dissemination /Exploitation is also up on track. The first results are coming in for WP2 and the risk is moderate.

**2. Ethical Issues**

The animal experiments were performed by the Ukraine partner in line with ethical guidelines of their institution and as provided to the EU before the start of the project. No other ethical issues have emerged during this period.

**3. Additional information**

Concerning disseminations activities, most of the activity was devoated to the publication of peer-reviewed articles in high standard journal. The PANG project has currently resulted in eight papers, one of them in the highly known journal of the Proc. Natl. Acad. Sci. U S A. 2016 Oct 4;113(40):E5856-E5865. The paper entitled “Affinity of Glycan-modified nanodiamonds towards lectins and uropathogenic *Escherichia Coli*” showing an innovative strategy for the covalent integration of any type of oligosaccharides without loss in specific interaction with lectins and pathogens has been selected by ChemNanoMat, Wiley, as hot articles and the topic was presented in an inside cover page

Regarding Outreach activities, two workshops have been organised since the start of the project. One, being in addition, the Kick off meeting of PANG, on “Nanomaterioals and Antibiotics (London, UK), the other recently in Berlin with the topic “Carbon-based nanoheaters: Immunogenic Cell Death of Solid Tumors” To this can be currently added two conferences one at the E-MRS Spring Meeting, one at the Hickinbottom meeting in London.

The secondements of Madame Wang Qian (USTL) was also helpful for her being recruited as Assistant Professor at the University of Jinan, Shandong, China.

Furthermore, due to the involvement in PANG and being leader of the LNMU team, Dr. Rostyslav Bilyy was currently promoted as full Professor.

**Publications**

1. K. Turcheniuk, T. Dumych, R. Bilyy, V ; Turcheniuk, J. Bouckaert, V. Vovk, V. Chopyak ; V. Zaitsev, P ; Mariot, N. Prevarskaya, R. Boukherroub, S. Szunerits

Plasmonic photothermal cancer therapy with gold nanorods/reduced grpahene oxide core/shell nanocomposites

RSC Advances 2016, 6, 1600

2. S. Szunerits, R. Boukherroub

Antibacterial activity of graphene-base materials

Journal of Material Chemistry B, 2016, 4, 6892

3. V. Turchenik, K. Turcheniuk, J. Bouckaert, A. Barras, T ; Dumych, R. Bilyy, V. Zaitsev, A. Siriwardena, Q. Wang, R. Boukherroub, S. Szunerits

Affinity of Glycan-modified nanodiamonds towards lectins and uropathogenic Escherichia Coli

ChemNanoMat 2016, 2, 307-314 (inside cover)

4. S. Szunerits, O. Zagorodko, V. Cognez, T; Dumych, T; Chalopin, D. Alvarez Dorta, A. Sivignon, N. Barnich, A. Harduin-Lepers, I; Larroulet, A. Yaguas Serrano, A. Siriwardena, A. Pesquera, A. Zurutuza, S. G. Gouin, R. Boukherroub, S. Szunerits

Differentiation of Crohn’s Disease-Assocaited Isolation from Other Pathogenic Escherichi coli by Fimbrial Adhesion under Shear Force

Biology, 2016, 5, 14

5. F. Teodorescu, G. Quéniat, C. Foulon, M; Lecoeur, A. Barras, S; Boulahneche, M. S. Medjram, T. Hubert, A. Abderrahmani, R. Boukherroub, S. Szunerits

Transdermal skin patch based on reduced graphene oxide: A new approach for photothermal triggered permeation of ondanestron across porcine skin

Journal of Controlled Release, 2017, 245, 137-146

6. F. Teodorescu, Y. Oz, G. Quéniat, A. Abderrhamani, C. Foulin, M. Lecoeur, R. Sanyal, A. Sanyal, R. Boukherroub, S. Szunerits

Photothermally triggered on-demand insulin release from reduced graphen oxide modified hydrogels

Journal of Controlled Release, 2017, 246, 164

7. M. H. C. Biermann, M. J. Podilska, J. Knopf, C; Reinwald, D. Weidner, J. Hahn, C. Maueroder, D. Kienhofer, A. Barras, M. Hoffman, R. Boukherroub, S. Szunerits, G. Schett, R. Bilyy, Y. Zhao, M. Hermann, L. E. Munoz

Oxidative burst-dependent NETosis is implicated in the resolutiojn of necrosis-associated sterile inflammation

Frontiers in Immunology (section: Molecular Innate Immunity) 2016, 7, 557

8. Muñoz LE, Bilyy R, Biermann MH, Kienhöfer D, Maueröder C, Hahn J, Brauner JM, Weidner D, Chen J, Scharin-Mehlmann M, Janko C, Friedrich RP, Mielenz D, Dumych T, Lootsik MD, Schauer C, Schett G, Hoffmann M, Zhao Y, Herrmann M.

Nanoparticles size-dependently initiate self-limiting NETosis-driven inflammation.

Proc Natl Acad Sci U S A. 2016 Oct 4;113(40):E5856-E5865.

**Workshops**

**1. Nanomaterials and Antibiotics**

30th November 2016-1sd December 2016

Sponsored by French Embassy London

Organisers: Sabine Szunerits (USTL partner) and Mike Watkionson (Queen Mary, London)

Objectives

1. Kick off meeting of PANG
2. Bring together specialists and researchers in the UK and outside interested in the understanding of glycan-pathogen interactions and in the development of alternative approaches for fighting against bacterial infections

**2. Carbon-based nanoheaters: Immunogenic Cell Death of Solid Tumors**

Thursday 23sdFebruary 2017-24th February 2017

Sponsored by French Embassy in Berlin (4000 Euros)

organisers: Luis MUNOZ (partner 3) and Sabine Szunerits (partner 1)

**Conferences**

1. Sabine Szunerits, 10th Hickinbottom meeting, QMW, London, UK, **2016,** Skin patches for the treatment of diabetics
2. Sabine Szunerits, Julie Bouckaert, E-MRS, Spring Meeting, Symposium J, Lille, **2016**, Plasmonic nanostructures for the inactivate  of bacteria strains