Periodic Report II
M18 – M36

Grant Agreement number: 211436

Project acronym: EMBEK1

Project title: Development and analysis of polymer based multifunctional bactericidal materials

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Declaration by the project coordinator

I, as co-ordinator of this project and in line with my obligations as stated in Article II.2.3 of the Grant Agreement declare that:

- The attached interim report represents an accurate description of the work carried out in this project for this activity period;
- The project (tick as appropriate):
  - X has fully achieved its objectives and technical goals for the period;
  - ☐ has achieved most of its objectives and technical goals for the activity period with relatively minor deviations;
  - ☐ has failed to achieve critical objectives and/or is not at all on schedule.
- The public Website is up to date, if applicable.
- To my best knowledge, the financial statements which are being submitted as part of this report are in line with the actual work carried out and are consistent with the report on the resources used for the project (section 3.6) and if applicable with the certificate on financial statement.
- All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under section 5 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

Name of Coordinator: .Dr. R. Förch.

Date: 31/ 07/ 2011

Signature of Coordinator: .....

2. PROJECT OBJECTIVES AND ACHIEVEMENTS DURING THE REPORTING PERIOD II

2.1. MILESTONES FOR REPORTING PERIOD II (M18-M36)

Table 1: The milestones for this reporting period M18-M36 were as follows:

Milestone no.	Milestone name	WP involved	Deliverables	Beneficiary	Due date from Annex I	Achieved Yes/No	Comments
8	2nd generation of antimicrobial coatings	WP1	D24	P1,P2,P3	M24	Yes	
9	Lab scale demonstrator	WP1	D30	P1,P2,P3	M24	Yes	
10	Improved analytical methods	WP2	D25 D26 D27	All Ps	M24	Yes	
11	2nd generation bacterial mutants and tests, insect model	WP3	D28 D32	P2 P4 P6	M24	Yes	
12	Preliminary catalogue of antimicrobial coatings	WP5	D35	All Ps	M30	Yes	
13	Modified demonstrator/ prototype	WP1 WP5	D37	P1 P2 P3 P7-P10	M30	Yes	
14	Insect model – long term effects	WP3	D38	P2	M30	Yes	
15	Sophisticated theoretical model of bacterial attachment on different length scales	WP4	D36	P1	M30	Yes	
16	Catalogue of antimicrobial coatings	WP5	D42 D45	All Ps	M36	Yes	
17	Coated prototype	WP1 WP5	D43		M36	YES	
18	3rd generation bacterial mutants, genotyping and proteomic analysis	WP3	D40, D41	P2 P4 P6	M36	YES	
19	Feasibility study	WP2 WP5	D44, D45	All Ps	M36	YES	

2.2. DELIVERABLES FOR REPORTING PERIOD II (M18-M36)

Table 2: The deliverables for this reporting period M18-M36 were as follows:

No.	Deliverable name	WP No.	Bene-ficiary	Nature	Dissemination level	Delivery date	Delivered Yes/No	Comment
D24	2nd generation of coatings with optimised antimicrobial properties	WP1	P1,P2, P3	D/R4	RE	24	Yes	
D25	Report on methods for determining antimicrobial properties	WP2	P1,P2, P5, P6, P8	R4	PU	24	Yes	
D26	Report on methods for stability and persistence of properties	WP2	All Ps	R4	RE	24	Yes	
D27	Impidemetric assay with 2nd generation of bacterial mutants and coatings	WP2	P5	R4	RE	24	Yes	
D28	2nd generation micro-evolved bacterial mutants	WP3	P2,P4, P5, P6	D	RE	24	Yes	
D29	Basic model of bacterial resistance and virulence	WP3	P2,P4, P5, P6	R4	RE	24	Yes	
D30	Demonstrators (laboratory samples) showing different levels of antimicrobial persistence	WP1	P1,P2, P3	D	RE	24	Yes	
D31	Predictive theoretical models of the abiotic-biotic interaction	WP4	P1, P3, P4	D/R4	RE/PU	24	Yes	
D32	Insect model - Mid term effects of developed coatings	WP3	P2	D/R4	RE/PU	24	Yes	
D33	Publications & IPR issues	WP7	All Ps	O	PU	24	Yes	
D34	**Interim report R4**	**WP6**	**P1**	**R4**	**CO**	**24**	Yes	
D35	Preliminary catalogue of antimicrobial coating with different levels of antibacterial persistence	WP5	All Ps	D	PU/RE	30	Yes	

D36	Sophisticated predictive models of abiotic-biotic interaction based on different length scales and concepts	WP4	P1, P2, P3, P4	D/R5	PU/RE	30	Yes	
D37	Small scale demonstrators/ prototypes	WP1 , WP2, WP3	All Ps	D/P	PU/RE	30	Yes	
D38	Insect model – long term effects & evaluation of insect study	WP3	P2	D/R5	PU/RE	30	Yes	
D39	**Interim report R5**	**WP6**	**P1**	R5	CO	30	Yes	
D40	3rd generation micro-evolved bacterial mutants	WP3	P2,P4,P6	D/R6	PU	36	YES	
D41	Identification of differences in protein expression levels in bacteria evolved to survive antimicrobial surfaces	WP3	P6	R3	PU	36	Yes	
D42	Final catalogue of antimicrobial coating with different levels of antibacterial persistence	WP5	All Ps	P	PU/RE	36	Yes	
D43	Small scale prototypes with optimized antimicrobial coating	WP5	All Ps	P,R6	PU/RE	36	YES	
D44	Assessment of developed metrology	WP2	All Ps	O,R6	RE/PU	36	YES	
D45	Feasibility study of developed materials	WP5	All Ps	O,R6	RE/PU	36	YES	
D46	Publications & IPR issues	WP7	All Ps	O	PU/RE	36	YES	
D47	**Interim report 6 (to coordinator)**	**WP6**	**All Ps**	**R6**	**CO**	36	YES	
D48	**Final report (to commission) – year 1-3**	**WP7**	**P1**	**FR2**	**CO**	36		

INTERIM ACTIVITY REPORT VI
M30 – M36

Grant Agreement number: 211436

Project acronym: EMBEK1

Project title: Development and analysis of polymer based multifunctional bactericidal materials

Funding Scheme: CP-FP7-NMP-2007-SMALL-1

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PUBLISHABE EXECUTIVE SUMMARY FOR THE
Interim Activity Period M30-M36
EMBEK1, grant# 211436

This final interim activity period for EMBEK1 has involved the evaluation of materials, processes and methods developed during the EMBEK1 programme which are associated with the following deliverables and milestones :

(i) finalising the microevolution of bacteria as a result of the materials developed in this programme by identifying differences in protein expression levels and genetic mutations in *S. aureaus, P. aeruginosa* and closely related strains – D40, D41, milestone 18

(ii) The set up of a catalogue of antimicrobial surfaces developed in this program with different levels of antimicrobial persistence. Preparation of small scale demonstrators and prototypes using these developed coatings and feasibility study for upscaling and further development. - D42, D43, D45, milestones 16, 17, and 19

(iii) Assessment of the developed metrology – D44

The work over the past 6 months has aimed at finalising the individual smaller projects within EMBEK1 in order to fulfil the deliverables and milestones of the programme. This includes the Zinc-release coatings, the Ag-release coatings, bilayers of Ag/Zn-release layers, the Zinc Schiff bases, the Cu-release coatings as well as ZnO-nanoparticle containing polymers and surface immobilized bacteriophages. The work has resulted in a number of publications which have already appeared in the literature, that are in the review process or are currently being written. Three patents have resulted from the development of new materials within EMBEK1. During the final months selected optimized coatings have been transferred into the preparation of demonstrators and prototypes. It could be shown that the coatings may be applied to textile materials, polymeric and metallic implants as well as to the inside of small tubing for possible applications in catheters. The results of these studies are presented in the M30 and M36 Interim activity reports. The Ag-release systems could be transferred to a pre-pilot scale plasma reactor and 60 cm wide textile material could be successfully coated by the partner EMPA. Up-scaling the Zn-release systems will still require further engineering development. The materials developed

and tested during the programme have been catalogued and are available as part of the final report.

Microevolution of the bacteria has probably been the most lengthy and cumbersome challenge, and the bacterial strains tested did not develop resistance towards the silver containing materials tested. This has shown in several independent studies within the programme and is one of the main advances in knowledge emanating from the EMBEK1 programme. However, resistance towards copper was achieved. Exposure of *P. aeruginosa* to the Zn-release systems could be shown to lead to an overexpression of enzymes responsible for coping with oxidative stress; enzymes related to such mechanisms could be identified and backed up by physiology data. We thus conclude that up regulation of these enzymes during degradation of the organo-chemical part (ligand) of the Zn- release system lead to oxidative stress.

We have developed a method to compare the full transcriptional profile of bacteria grown under different conditions using RNA-seq. The transcriptomics results have revealed that there are very few differences in mRNA abundance in response to the different host temperatures. Temperature is a key signal of the host environment for many pathogenic bacteria. Our results indicate that *P. asymbiotica* responds to insect infection temperature (28°C) in a similar way to that of human body temperature (37°C), with no obvious differences in virulence gene expression. In addition to this, the transcriptomic analysis of *P. asymbiotica* has provided an unprecedented level of molecular detail of transcriptional start and stop sites, operon structure, novel putative protein coding genes and small non-coding RNA (sRNA) that were previously not annotated in the genome. When analysis of this data is completed it will be made available at a bespoke web site (hosted at the University of Bath) and will provide an invaluable resource to the scientific community.

The partners met on 20-21. June for a final project meeting at the partner MPIP in Mainz, Germany, where all partners participated and reported on the progress during the final 6 months. The meeting ended with a gathering of the scientific project committee (SPC) where the overall achievements of the project were discussed and the final conclusions of the work within EMBEK1 were summarised. While the EMBEK1 programme has finished successfully, the SPC participants could identify several areas where follow-up projects would be required to answer questions that have evolved during EMBEK1. Continued collaborations on various levels are anticipated between a number of the partners.

SECTION 1 –

PROJECT OBJECTIVES AND ACHIEVEMENTS DURING THE INTERIM ACTIVITY PERIOD VI

1. MILESTONES

Table 1: The milestones for the interim activity period M30-M36 were as follows:

Milestone no.	Milestone name	WP involved	Deliver-ables	Beneficiary	Due date from Annex I	Achieved Yes/No	Comments
16	Catalogue of antimicrobial coatings	WP5	D42 D45	All Ps	M36	Yes	
17	Coated prototype	WP1 WP5	D43		M36	YES	
18	3rd generation bacterial mutants, genotyping and proteomic analysis	WP3	D40, D41	P2 P4 P6	M36	YES	
19	Feasibility study	WP2 WP5	D44, D45	All Ps	M36	YES	

2. DELIVERABLES

Table 2. Deliverables for the interim activity period M30 to M36

No.	Deliverable name	WP No.	Bene-ficiary	Nature	Dissemination level	Delivery date	Delivered Yes/No	Comment
D40	3rd generation micro-evolved bacterial mutants	WP3	P2,P4,P6	D/R6	PU	36	YES	
D41	Identification of differences in protein expression levels in bacteria evolved to survive antimicrobial surfaces	WP3	P6	R3	PU	36	Yes	
D42	Final catalogue of antimicrobial coating with different levels of antibacterial persistence	WP5	All Ps	P	PU/RE	36	Yes	
D43	Small scale prototypes with optimized antimicrobial coating	WP5	All Ps	P,R6	PU/RE	36	YES	
D44	Assessment of developed metrology	WP2	All Ps	O,R6	RE/PU	36	YES	
D45	Feasibility study of developed materials	WP5	All Ps	O,R6	RE/PU	36	YES	
D46	Publications & IPR issues	WP7	All Ps	O	PU/RE	36	YES	
D47	**Interim report 6 (to coordinator)**	**WP6**	**All Ps**	**R6**	**CO**	36	YES	
D48	**Final report (to commission) – year 1-3**	**WP7**	**P1**	**FR2**	**CO**	36	YES	