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PROJECT PERIODIC REPORT- Publishable summary

InFact

Functional materials for fast diagnosis of wound infection

NMP.2013.4.0-3

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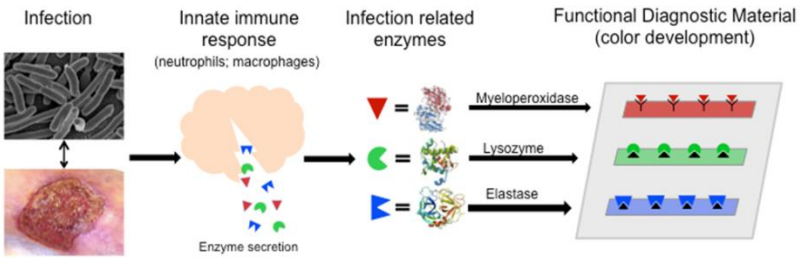
Project website: http://in-fact.eu/

PUBLISHABLE SUMMARY

InFact project, aims to develop and clinically validate a Point of Care Testing (PoCT) device to identify incipient wound infection.
Infection is a global problem of traumatic, post-surgical or chronic wounds. 10% of surgical wounds exhibit bacterial infection within 30 days. Heavy bacterial colonization is the main reason for non-healing of chronic wounds such as decubitus, ulcus cruris and diabetic foot ulcers. Early detection of an incipient wound infection is important for the attending physician, since it would allow the timely initiation of treatment, thus reducing the severity of the disease . Currently, however, wound infection is not diagnosed until becoming pathologically evident. As a consequence, the treatment of the patient is further complicated and more likely to have a negative outcome. In addition, wounds are often treated with antibiotics prophylactically, leading to unnecessary selection for bacterial resistance.
Consortium partners have patented the know-how to convert wound dressings into a diagnostic tool capable to inform both patient and therapist about the wound status, thus allowing a proactive diagnostic step. The proposed functional materials allow a real time *in situ* infection diagnostic reaction and, thus, a timely treatment intervention. A next-generation Protective, Predictive and Proactive (triple-P) material for in situ diagnosis of wound infection to be integrated in conventional dressings will be prototyped and advanced to commercialization by InFact.
During the first period technical RTD WPs 1-6 were active.



1st period results:

WP1 Optimization of color reaction between enzyme substrates and the wound infection enzymes (myeloperoxidase, lysozyme and elastase) (Leader: BOKU)

During the first project period the best combination of phenolic substrates to be used for MPO detection were screened and validated (Task 1.1). By the end of the 18th month several substrates, alone and in combination, have been tested and 6 mixtures were chosen for their distinctive colour. These combinations of MPO substrates have been sent to BIU for sonochemical immobilization on gauzes. After coating the gauzes with the substrates UPC determined the MPO oxidation of the permanently immobilized on the gauze substrates and upon their release (loosely immobilized substrates) from the fabric. In parallel, a strategy to capture MPO in the detection window by using specific antibodies was also investigated following the idea to provide a revealing kit containing the above MPO substrates.
In order to reach the goal of the task 1.2 - to synthesize a substrate for the detection of elevated lysozyme activities in wounds based on the biomaterial chitosan (a compound extractable from non-animal sources), during the first 18M three substrates were successfully prepared based on N-acetyl chitosan that was stained with a certain dye. All substrates enable a visual detection of lysozyme activity only applying low amounts of the substrate. The substrates are currently under investigation for the incorporation into the PoC testing device.
The main goal of the task 1.3 is to synthetize chromogenic peptides with high affinity for HNE for further incorporation into a wound dressing. By the end of the first year, we have successfully selected the peptide specific cleavage sequence for HNE, Alanine-Alanine-Proline-Valine (AAPV) using molecular docking simulations and *in vitro* HNE kinetics studies. Other strategies are currently under development.
Within task 1.4 strategies for the incorporation of an internal standards are discussed whereby several dyes are currently examined being suitable candidates as an internal standard.
The main goal of the task 1.5 is the identification of additional enzymes that can be detected in wound infection. Among a potential pool of enzymes, phospholipase C and phospholipase A2 could be identified showing elevated activities in several wound fluids from infected wounds. Within this task, enzyme assays were additionally developed to reproducibly detect the found enzymes.

WP2 Lab prototype production and characterization of the 'in situ wound infection diagnostic material' (Leader: BIU)

One of the tools in forming the diagnostic material is surface coating by substrates that are known as suitable for the detection of wound's enzymes. In the current project, sonochemical coating technique is one of the suggested approaches for depositing the material. The coating of the sensing material that is formed has a nanostructure. The sensing nanoparticles are synthesized from water solution in a one-step process. The molecules that are deposited on the surface are suggested by the consortium partners. Some of the sensing molecules are commercially available and some are developed within the project. Meanwhile, very promising results are obtained with substrates for detection of MPO. A good colour response was achieved in combining 2 MPO different substrates, for example 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS), with 4-Hydroxybenzoic acid (PHBA).

WP3. *In vitro/in vivo* assessment of the 'in situ wound infection diagnostic material' (Leader: SYN)

The *in vitro* testing of the materials and the devices is conducted at various levels from a liquid phase enzymatic reaction, to a solid phase reaction in which the materials are placed onto a support and that is wet with a test solution, to then a solid support that is wet with an actual wound fluid. Experience in test suggests that the overall properties of the system are best replicated by a full prototype because it is only in these circumstances that the variables of substrate adhesion, reaction, compatibility of materials, glues and binders are fully assessed in parallel.

Within in vivo testing, the main criterion for safety in clinical use is the property of separation between the device components and the wound surface. The key goal of developments in this case is to ensure that no components of the detection level can be leached back toward broken skin. To this end, the main goal of in vivo testing is to observe and detect whether the water vapour generated by the skin which must travel through the dressing results in any back permeation or other moisture transfer to the contact layer of the dressing.

WP4. In human clinical studies: *ex-vivo* and *in vivo* (Leader: MST)

Work package 4 consists of three clinical efficacy studies in humans (ex-vivo and in-vivo), which will be performed during M24-M48. Within the first study, ***ex-vivo* study 1**, the enzyme substrates for myeloperoxidase (MPO), lysozyme and human neutrophil elastase (HNE) will be tested with 200 wound exudate samples. The aim of this study is to determine the diagnostic properties of the enzyme substrates for the detection of wound infection, with microbiological culture of wound swabs as gold standard. This study is planned for month 24 – 33. Within the first period (month 1-18), MST has prepared the infrastructure for this study. This entails setting-up collaborations with various departments and home care for the conduction of this and future studies. Conducting these clinical studies in both hospital and home care setting, instead of only the department of surgery of MST, better represents the future target population for the InFact dressing. In addition, MST is also working on a study, which compares the microbiological culture results of wound biopsy (gold standard for wound infection according to literature) and wound swab. This study must provide insight in whether wound swabs are sufficient as gold standard for these clinical studies. The rationale behind this is the fact that wound biopsies are logistically difficult to perform and patients are often reluctant to undergo wound biopsies because of pain or fear of deterioration of the wound. Wound swabs on the other hand are easy to perform and usually painless for patients. The second study, ***ex-vivo* study 2**, will follow-up on the first ex-vivo study; the enzyme substrates are now incorporated in the wound dressing material. To assess whether the enzyme substrates are still functional while incorporated in the material, the material will be tested (ex-vivo) with wound exudate collected from 10 patients with an infected wound and 10 patients with a non-infected wound. MST aims to expand this study, if possible, with the aim to determine whether there is no loss in diagnostic properties of the enzyme substrates once they are incorporated in the wound dressing. This expansion is only possible if the wound fluid samples from ex-vivo study 1 can also be used for testing during ex-vivo study 2. The third, and last, study will consist of an ***in-vivo* clinical study**. Within this study the InFact wound dressing will be tested in patients, in both hospital and home care setting. The primary end-point(s) of this study are the diagnostic properties of the InFact material, with microbiological culture results of wound swabs as gold standard. In addition, Convatec will try to extract market relevant information during the studies such as on handling issues for further optimization of the product.

WP5 Up-scaling, LCA and LCC studies and process engineering for mass production (Leader: QZY)

The goal for the first 18 months was to set up an environmental evaluation and an economic evaluation of a prototype of the diagnostic wound dressing being developed in InFact. In order to avoid sub-optimization and to give insight in the relative importance of e.g. costs and environmental aspects of material production in the complete treatment scheme, the initial scope of 'prototype production' has been broadened towards treatment of the patient. This has so far led to the insight that in case of no infection, InFact dressings lead to no significant differences in the environmental impact. The largest contributors are the use of transportation by the home care nurse, followed by the manufacture of the bandages that are used to fix the dressing. In case of an infection, the absolute environmental impact increases significantly, because the patient needs additional care in e.g. an outpatient clinic. The application of InFact dressings then can reduce the environmental impact when early detection leads to quicker healing. In terms of CO_2 emissions, this can save 20-25% compared to treatment with traditional dressings. Critical is the number of outpatient visits as well as the energy consumption of the outpatient clinic.

WP6 Towards CE marking, market survey and business plan (Leader: CON)

During the first period consultation with BSi/MHRA has been undertaken to determine the likely regulatory pathway in Europe.

In-house research with clinical colleagues has been undertaken to determine clinical needs and product design options, with a view to developing a customer-driven product. However, further external research is still required to verify or modify internally-specified requirements. An initial business plan has been considered although significantly more input is required that will be determined by feedback from market research, material and manufacturing costs etc.

WP 7 Dissemination, Training and Exploitation (Leader: SYN)
WP7 is focusing on training, dissemination and exploitation and is activated from beginning of the project. All partners are contributing to these activities. An interactive project website has been launched to share knowledge and data inside and outside the consortium (http://in-fact.eu/)

Presentations and posters in Conferences:
Several partners have presented the project concepts at conferences and as invited lectures:

- **MST:** European Wound Management Association Conference 14-16th of May, 2014, Madrid (Spain), http://ewma2014.org; Congres Wondgenezing en Wondbehandeling (Conference Wound Healing and Wound Management), 12th of December, 2014, Rotterdam (The Netherlands) http://www.wondcongres.nl/?q=node/2
- **MST, QZY** European Wound Management Association Conference 13-15th of May, 2015, London (United Kingdom), http://www.ewma2015.org
- **BIU:** "Coating antibacterial NPs on flat and curved Surfaces" Plenary lecture at the ICAFM conference at Trivandrum , India, Feb. 20, 2014; "Coating antibacterial NPs on flat and curved Surfaces" Goa University, India, Feb. 24, 2014; "Sonochemical coating" Lecture at MIRDC. Kaohsiung Taiwan March 5, 2014; "Coating Anti Bacterial, Anti Viral, Antibiofilm and Antifungi;Nanoparticles on Flat and Curved Surfaces Employing the Sonochemical Method" Lecture at Tzhjiang University Madical Hospital Number 2, April 28, Hangzhou, China

Impact:
In the western industrialized countries, about 2% of the population suffers from chronic wounds. Decubitus wounds, ulcus cruris and diabetic feet are the most abundant types of chronic wounds, mostly associated with age. Demographic trends show a significantly increased life expectancy, combined with strong growth in chronically ill and dependent people. 25% of these chronic wounds become infected once a year. In addition, 10% of postsurgical wounds are infected within 30 days, leading to additional costs of 23 Mrd. € per year in Europe. Patients with wound infection require an additional median duration of 6.5 days in hospital resulting in doubling the hospital costs**Error! Bookmark not defined.**. The annual total costs for treating infected wounds **exceed 5 billion € per year in Europe** placing a substantial financial burden on the health care system. The magnitude is expected to increase as the population ages. Consequently, early diagnosis of wound infection is of tremendous importance to improve both the current societal impacts and economic consequences.
InFact answers directly to the topic '*NMP.2013.4.0-3 From research to innovation: substantial steps forward in the industrial use of European intellectual assets, stimulating the use of newly developed materials and materials technologies by the industry*' by **transforming the novel scientific results** obtained by some of the consortium partners in the previous European LIDWINE**Error! Bookmark not defined.** towards products with high market potential. Furthermore InFact is based on two patents with the **European Patent Office (EPO)** EP 09160557.6 and PCT/AT2011/000074. Though the development of functional materials with diagnostic function **InFact will 'build' the required bridge** over the **'valley of death'** from scientific results to useful products.
The **advantages of the InFact technology,** compared to existing methods such as microbiological analysis after taking swab samples, is a much faster diagnostic response (only minutes compared to days) and the simplicity of the technology (simple colour reaction of materials compared to instrumental analysis). For the first time functional materials for wound infection detection will become available at affordable cost for a wide use both in home care and in hospitals. This will be feasible based on the integration of the diagnostic function in materials already commonly used in wound dressings.
The diagnostic materials developed in InFact have a **large socio economic impact and market potential related to wound management**, both in home care and hospitals.
Novel approaches to detect or prevent infection are one of the most important medical issues. InFact proposes capitalising on this leading IP position to deliver a revolutionary product to wound care.
The realization of this product will effectively contribute, to the transformation of the **European biotechnology and medicine** as well as the relevant industries from a resource-intensive to a knowledge-intensive phase, which will further contribute to the EU WW position in these fields. This project is part of the chain in this transformation, introducing high added value technologies in a sustainable manner. This project is at the crossroads between different disciplines and technologies (chemistry, biology nanotechnology, engineering and wound biology). The outputs of the project will increase European scientific and technological qualities, thus preventing relocation of European science to other areas worldwide, and at the same time creating industrial and employment growth within Europe.