

InFact project, aims to develop and clinically validate a Point of Care Testing (PoCT) device to identify incipient wound infection.

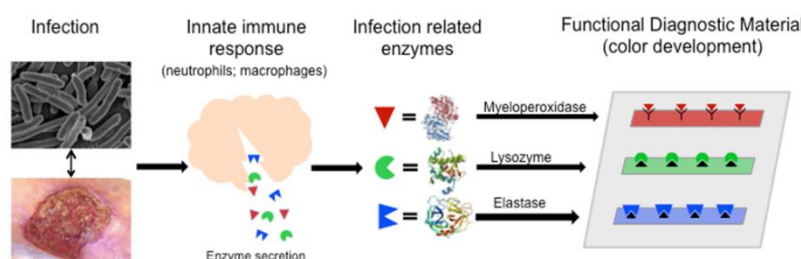
Infection is a global problem of traumatic, post-surgical or chronic wounds. 10% of surgical wounds exhibit bacterial infection within 30 days. Heavy bacterial colonization is the main reason for non-healing of chronic wounds such as decubitus, ulcer cruris and diabetic foot ulcers. Early detection of 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.



2nd period results:

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

The main goal of the Task 1.1 is to find the best combination of phenolic substrates to be used for MPO detection. 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.

Combinations of commercially available molecules have been assessed as MPO substrates. These compound combinations were selected based on their chromogenic properties considering the distinctive colours dark blue, dark green, violet and deep purple together with a clear transition between the non-oxidised and the oxidised state. At the same time and with the aim to achieve a substrate immobilization onto the surface of the diagnostic material the hydrophobicity and the presence of functional groups that would allow a chemical crosslinking upon MPO oxidation were desired properties. As a proof of concept, paper strips were used to assess the substrate colour change and immobilization

The main goal of the task 1.2 is 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. Within 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 synthesize 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'

The sonochemical coating technique is one of the approaches that were used for depositing substrates which can react with wound's enzymes. BIU has developed a coating procedure for immobilization of various substrates on the fabric. The coating that is formed has a nanostructure and it is synthesized in a one-step process in which the desired nanoparticles are formed and subsequently deposited on the surface. For detection of MPO the following molecules were used: Dianisidine (FBB), 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS), and 4-Hydroxybenzoic acid (PHBA). For Lysozyme detection, chitosan based compounds stained with a dye were synthesized by BOKU, and further sonochemically coated by BIU. For Elastase, 3 commercially available peptides were suggested as potential substrates and they were sonochemically coated on fabric. Optimization of reaction parameters was done and a good coating, in terms of enzyme activity, was obtained. The results have revealed that the best candidate for the detection of MPO is a combination of ABTS and PHBA that were simultaneously coated on fabric. This combination gave a strong blue colour response, when exposed to MPO solution. Succinyl chitosan stained with RB5 was successfully deposited on cotton and upon reaction with Lysozyme solution; a strong blue colour was developed. Elastase was detected by a fluorogenic substrate, which was sonochemically deposited on cotton. All the coated fabrics were characterized by electron microscopy, elemental analysis, and spectroscopy studies. The substrates that have demonstrated a good activity *in-vitro* will be further tested for their stability and activity in wound fluids.

WP3. In vitro/in vivo assessment of the 'in situ wound infection diagnostic material'

WP3 consists of the testing of the in-vitro efficiency, the in vitro biocompatibility and the in vivo biocompatibility, leaching and efficacy. During the 2nd period a theoretical risk assessment of the toxicology of the components of the indicator chemistry was done (CON). In the next step cell culture assays of the indicator chemistries followed by cell culture assays with the finally formulated inks were performed (SYN). The toxicological risk assessment was conducted to calculate the permissible device levels based on their individual safety assessment. Risk assessment methodology applied herein, as it applies specifically to medical devices, is described in ISO 10993-17:2002/(R):2012. For comparison, however, an alternative method was applied because limited human data were available. One example of an alternative method is the Acceptable Daily Exposure (ADE), or an Acceptable Daily Intake (ADI),¹ which appears in Chapter 5 of the 2010 ISPE RiskMaPP Guidance.

The cell culture MTT assay is a commonly used colorimetric assay to evaluate cell metabolic activity.

WP4. In human clinical studies: ex-vivo and in vivo

Ex-vivo study 1, in which the enzyme substrates were tested with wound fluid collected from patients at the wound care clinic, demonstrated that the use of enzyme assays is promising for the detection of wound infection. This enabled the partners to further develop the technology and incorporate it into sensors (InFact diagnostic material). These sensors will be tested during ex-vivo study 2. In this study MST will investigate whether the sensors are able to provide positive signals in wound dressings from infected wounds, and negative signals in wound dressings from not infected wounds. At this time, MST has finished the study protocol for ex-vivo study 2 and has started the collection and testing of wound dressings. In the upcoming period, ex-vivo study 2 will be completed, and the last in-vivo study will be intensively prepared and subsequently conducted in collaboration with partners ConvaTec, Synovo and Qualizyme.

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

The focus for the second period was upscaling the process for enzyme substrate production and synthesis. The synthesis / production of substrates for 3 different enzymes could successfully be optimised and up-scaled. Using these processes, substrates required for more than 100 InFact materials per day can be produced. Furthermore, stability and storage tests were started!

¹ ADI as used by CFSAN/FDA is 1/100 of the most sensitive NOAEL in chronic oral or reproductive toxicity study (no need for UF_R) expressed in mg/kg BW. In order for the material of interest to be considered safe, the estimated daily intake (EDI) expressed in mg/kg BW assuming 60-70 kg adult should be less than the ADI.

TNO continued to collect data for improving the current LCA/LCC.

WP6 Towards CE marking, market survey and business plan

A marketed wound dressing with infection status indication would be regulated as a Class IIB medical device in the EU. This is with the proviso that its primary function is a dressing, and that any additional chemicals are not available to the wound nor are of animal origin. The device Technical File will require review by the Notified Body. US regulatory route is to be determined. While no predicate infection-indicating dressings are on the market, a protease diagnostic test card device is available in the EU, Canada and Australia, and a second generation bacterial protease diagnostic test card device is anticipated in the EU in 2017.

Global market research is in-progress to guide product format options, barriers to market entry, price and volume potential.

An initial business plan was submitted in M24, covering the competitive landscape, the collaborative product development status, risks and opportunities, and initial regulatory and financial analyses. Future launch plans will be generated for global, regional and country launches, alongside required financial plans and post-launch monitoring plans.

WP 7 Dissemination, Training and Exploitation

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/>)

Dissemination

Project website has been launched to share knowledge and data inside and outside the consortium (<http://in-fact.eu/>)

Patents

Three patents were submitted including inventors from **SYN, QZY, BOKU** and **CON**.

1. Detecting Microbial Infections in wounds - Novel Dyes for diagnostics – submitted 2016 – US 62/315,546
2. Detecting Microbial Infections in wounds - Novel device and substrates – submitted 2016 - US 62/315,565
3. Detecting Microbial Infections in wounds - dressings – submitted 2016 – US 62/315,556

The applications cover reactive substances for use in diagnostic products, devices for using those substances and methods of incorporating them in wound dressings. The applications are at the provisional phase.

Publication s in peer reviewed journals

1. Gregor Tegla, Alexandra Rolletta, Jasmin Dopplinger, Clemens Gamerith, Georg M. Guebitz, Chitosan based substrates for wound infection detection based on increased lysozyme activity, **Carbohydrate Polymers**, **2016**, 151, 260–267 (doi:10.1016/j.carbpol.2016.05.069)
2. Gregor Tegl, Christoph Öhlknecht, Robert Vielnascher, Alexandra Rollett, Andreas Hofinger-Horvath, Paul Kosma, and Georg M. Guebitz, Cellobiohydrolases produce different oligosaccharides from chitosan, **Biomacromolecules**, **2016**, 17 (6), pp 2284–2292 (DOI: 10.1021/acs.biomac.6b00547)
3. Myeloperoxidase responsive materials for infection detection based on immobilized aminomethoxyphenol, **Biotechnology and Bioengineering**, accepted
4. Ana V. Ferreira, Ilana Perelshtein, Nina Perkash, Aharon Gedanken, Joana Cunha, and Artur Cavaco-Paulo, Detection of Human Neutrophil Elastase (HNE) on Wound Dressings as Marker of Inflammation, **Appl Microbiol Biotechnol**, **2016**, (doi: 10.1007/s00253-016-7889-6)
5. Haalboom, M. Chronic wounds: Innovations in diagnostics and therapeutics. **Current Medicinal Chemistry**, submitted and under review

Presentations and posters in Conferences:

1. The importance of Defects in determining the antibacterial properties of metal oxide Nanoparticles" Lecture at **EMRS conference in Lille France**, May 3, 2016.
2. The future HOSPITAL a place where all its textiles will be antibacterial, A lecture at the Holon Inst. of Technology in the **NATI Symposium**, May 8, 2016, Holon, Israel.
3. The Sonochemical Coating: Making the HOSPITAL a Safer Place where all its textiles will be antibacterial, **NanoSMAT-2016 a conference, University of Texas, Arlington**, May 18, 2016.
4. Advances in Functional Materials, 29.6. to 3.7.2015 at Stony Brooks **University, Long Island, USA**

5. **BioNanoMed 2016**, 6.4.-8.4. 2016, **Krems, Austria**
6. Tzanko Tzanov, Kristina Ivanova, Petya Petkova, Eva Ramon, Antonio Francesko, Margarida Fernandes, Carlos Díaz Blanco, Antibacterial approaches in medical environment from materials engineering perspective – enzymes on work. **251st American Chemical Society National Meeting & Exposition, San Diego (USA)**; Oral presentation
7. Eva Ramon, Antonio Francesko, Tzanko Tzanov, Point of care device for detection of myeloperoxidase as a wound infection marker. **251st American Chemical Society National Meeting & Exposition, San Diego (USA)**; Oral presentation
8. Ana V. Ferreira, Joana Cunha, and Artur Cavaco-Paulo, Monitoring human neutrophil elastase (HNE) in chronic wounds. **251st American Chemical Society National Meeting & Exposition, San Diego (USA)**; Oral presentation

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 surgical site infection (SSI) require an additional median duration of 6.5 days in hospital and an attributable hospital cost of 2,780€²Error! 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.

Employment

With the aging population, **health care remains the go-to field for job growth**. "Health care is always adding jobs. That will clearly continue," says Dean Baker, co-director of the Center for Economic and Policy Research, a Washington think tank.

Among companies that expect to increase full-time, permanent workers in 2011, the top areas, by function, according to the survey are: sales, information technology, customer service, **engineering, technology**, administrative, business development, marketing, **research/development** and accounting/finance³. InFact is at the intersection of the above mentioned discipline thus having a positive impacting the employment in these fields.

Quality of life

Battling serious wound infection becomes a dominant factor in the lives of affected patients. Wound infection is painful, socially limiting and potentially lethal - thus it imposes dramatic psychological as well as physical burdens on patients. The prospect of wound infection is also a significant disincentive to undertake treatment of chronic injuries. Thus, a person who may ordinarily benefit from, say, a knee re-construction, may forego the treatment for fear (not-unreasonably) that a deep bone infection could render them less mobile than their pre-operative status.

Thus, steps to reduce rates of infection or otherwise more effectively deal with incipient infections will have a dramatic effect on both the lives of existing patients as well as increasing the confidence with which the general public undertakes medical treatment

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.

² Edward S. Wong , MD, The Price of a Surgical-Site Infection: More Than Just Excess Length of Stay Infection Control and Hospital Epidemiology, Vol. 20, No. 11 (November 1999), pp. 722-724

³ http://online.wsj.com/article/SB10001424052748704030704576070823246099088.html?mod=googlenews_wsj

Novel approaches to detect or prevent infection are one of the most important medical issues. **InFact** proposes capitalising on its 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.