TAMIRUT Project

EC Project Nr. NMP4-CT-2005-016382

The Project Status at end of P2

The project objectives

The overall objective of TAMIRUT is to develop an innovative bio-sensor concept devoted to advanced medical diagnosis, in which the biological material is carried by targeted micro-bubbles injected inside the body and the transducer is remote. The remote transducer operates on the basis of the ultrasound response of such micro-bubbles, gathered and processed by an improved version of an ultrasound medical scanner (UMS). Targeted micro-bubbles (similar to the generic micro-bubbles composing the contrast agents actually used in medical echography) are designed to bind only to a desired target region: a bioconjugate ligand is attached to micro-bubble shells producing the adhesion of them to specific molecular signatures. The project goal is to exploit targeted micro-bubbles as bio-sensors producing measurements that will significantly complement the ultrasound molecular imaging and improve the medical diagnosis potential.

Among many potential medical applications, the case study that will be addressed in the TAMIRUT project concerns the early detection, the assessment of the micro-vessel density, and the correct staging and grading of the prostate cancer. Such tasks will be dramatically improved by developing appropriate targeted micro-bubbles (i.e., bubbles able to bind on the endothelium of the neo-vessels of such a cancer) and measuring their local concentrations by means of the novel bio-sensor. In addition, the repetition over time of such a measurement could help in assessing the disease evolution, especially regarding the angiogenesis aspects.

The main objective of this project will be pursued by a multidisciplinary, strictly joint development of all the bio-sensor components, taking into account the need to remotely interrogate targeted micro-bubbles. In turn, micro-bubbles and bioconjugate ligands will be refined and tuned taking into account the desires of the medical community in relation to the introduction of new bio-sensor and addressed case study.

The main TAMIRUT expected output is the prototype of the described bio-sensor composed by the following components, each of which will constitute a partial result of the TAMIRUT project:

(1) **A Targeted UCAs** produced through advanced micro-bubble engineering, with ligand molecules having the efficacy to target specific endothelial markers (i.e., molecular signatures) which are over-expressed in the selected case study (prostate cancer).

(2) **Signal Processing Procedures** able to correctly excite the targeted micro-bubbles, detect and separate the echoes produced by the adherent micro-bubbles, derive the concentration of them for each resolution cell and with a defined tolerance.

(3) **An Ultrasound Medical Scanner** characterized by a great flexibility and a high computational power, able to accurately transmit waveforms of arbitrary shape, update the scanning scheme in real-time, and allowing, at the same time, traditional and harmonic imaging.

(4) **An Ultrasound Probe** able to linearly transmit and receive over an ultra-wide frequency band in order to assess (without any modification) the harmonic components due to the bubble non-linear scattering.

The consortium

The consortium is for a large part built on an already existing basis of co-operation in the field of ultrasound contrast imaging among **Esaote (I)**, a leading European ultrasound systems

manufacturer, and **Bracco Research (CH)**, one of the world's leading research centers in the field of micro-bubbles as diagnostic agents for ultrasound imaging.

This experimented partnership is here reinforced by the essential contribution of two SMEs as very expert industrial providers in the two specialist fields of probe construction, **Vermon (F)** one of the leading ultrasound probe manufacturer in the world, and of signal processing, **SignalGeneriX (CY)**.

The consortium is completed by contribution of scientific institutions with world-wide recognized expertise in their respective fields, like the **Fraunhofer IBMT (D)** in the field of bioanalytical chemistry and biotechnology, the **Department of Applied Physics of University of Twente (NL)** and the **Dept. of Biomedical Engineering of Erasmus Medical Center (NL)** in the field of physics of fluids for studying and modelling targeted micro-bubbles response to ultrasound excitation, the **Department of Biophysical and Electronic Engineering of University of Genova (I)** in the field of signal processing procedures and the **Dept. of Radiology of University of Innsbruck (A)** with its specialists of extensive experience in diagnosis and biomolecular research of prostate cancer, having the main role to specify and to evaluate the potential of the novel bio-sensor concept in medical diagnosis applications.

Activity up to end of period P2

After to have successfully reached at end of 1st year the objectives of the project dedicate to completion of user requirements and bio-sensor specifications and of scientific and technological implementation), during this 2nd year we get production and validation of the main components (targeted UCA, probe, scanner, signal processing software) of the novel bio-sensor system, and we are now ready to make use of if in the next integrationi and proof of concept of the novel biosensor.

In particular we perform a deep and extensive exploration of the physical concepts involved in the bubble modelling and the routines adopted to simulate the bubble response, giving origin to models used to derive the signal processing structure (in particular through developed simulation tool) and to assess the most probable (acoustic) characteristics for the targeted micro-bubbles structure under development. Thanks to the relevant analysis performed, TAMIRUT partners concluded that, from specifications stated for the possible UCA, it is not easy detectable a free bubble from an adherent bubble in a bubble population. Nevertheless it remain open the chance to use the wash out curve to detect presence of adherent bubbles population respect to a free bubbles population only. It is a less immediate but still valid method to demonstrate the effectiveness of TAMIRUT objective achievement: if targeted microbubbles echo will persist for long time means they adhere to the target and then the diagnosis of prostate cancer is positive otherwise their echo will vanish shortly and the diagnosis is negative. A similar point was considered more than sufficient whitin the purpose of TAMIRUT.

As consequence of it also the signal processing was adapted to the new situation to being able to detect the targeted microbubbles developed by BRACCO and IBMT, but compatible with the features and potentialities of the TAMIRUT's probe and scanner.

A stochastic method of synthesis able to produce an insonification beam having a quasi-constant pressure profile over a large depth interval has been devised and studied. It represents a tool that, if necessary, properly works for both single pulse emission and multiple pulses emission, disregarding the related processing algorithms.

The processing of the received signals, many different signal processing options, with a different degree of innovation and originality, have been considered. At the same time, the signal processing options that are can be used jointly with the refined ultrasound scanner and are compatible with the characteristics of the developed probe have been selected. At the end, a short list of signal processing options have been arranged that can be adopted for and integrated in the biosensor prototype, providing satisfactory performance in term of enhancement of the micro-bubble response against the tissue response.

A solution based on the regression implemented by a support vector machine (SVM) has been developed and tested using simulated data for the measurement of the bubble concentration. Despite the measurement of the bubble concentration revealed to be an original and very difficult task, the accuracy is quite in line with the needs of the envisaged medical application, although in absolute terms it is not very high.

In parallel to signal processing was studied and implemented the improved UMS (scanner and probe) showing specific functionalities concerning ultrasound waveform generation, transmission setting, signal grabbing and storage, memory space, computational power, and post-processing opportunities.

The improved scanner is derived from a basic high-end echographic platform from Esaote integrated with specific processing power to give to this platform the necessary high computational capability to run algorithms devised for the signal processing developed.

To tune/verify the algorithms with the necessary flexibility to analyse different signal processing options, the solution selected is an off line processing as the best way to be fast and flexible in testing evolving signal analysis of bio-sensor model without loosing capability of the system.

In last but not least fundamental we produced the refined ligand-bearing microbubbles and were selected and tested the possible antibodies for their possible use with the two targets previously chosen: PSMA and Tissue factor. Two methods for the formulation of ligand-bearing microbubbles have been developed. The first one, based on streptavidin-biotin interaction, allows testing of target antibodies on a small scale, the second method aims at producing the TAMIRUT ultrasound contrast agent on a larger scale. Two antibodies against Tissue factor were selected and tested during the period, #4509 from American Diagnostica and TF9-10H10 from Calbiochem. The antibody against PSMA selected is Abcam ab22335. From this individuated processes and solutions, microbubbles have been refined and are now available for further work with either anti-TF antibodies or anti-PSMA antibodies at their surface, the selected targeted UCAs as innovative bio-sensors of TAMIRUT are ready...

In conclusion, we can affirm that we are ready to start the next phase of integration and lab test of proposed biosensor, in perfect accord with project timing and planning.

Expected results, intentions for use and impact

Although experimentations on patients involving targeted-UCAs are not yet approved, the long-term philosophy of this project is implemented keeping in mind from now the (future) relevant medical applications for which the proposed bio-sensor represents a clear added-value: the development of novel ultrasound targeted micro-bubbles activated through remote ultrasound transduction is a very innovative approach to contribute in solving cancer diagnosis problems.

Cancer is a growing concern all over the world. Among the different kind of cancers, prostate cancer is the most frequent cancer among men accounting for 12% of all cancer cases overcoming also lung cancer accounting “only” for 10% of cases. Prostate cancer is still a rare event in men under 40’s but with a rate destined to increase with the age. It has been calculated that a man out of six is destined to experience prostate cancer clinically evident in its life. Most prostate cancers are multifocal, with synchronous involvement of multiple zones of the prostate, which may be due to clonal and nonclonal tumors. Unfortunately prostate cancer, for most part, is a silent disease for whom it is essential that specialist screening should be offered annually, beginning at age 50 years, to men who have at least a 10-year life expectancy and to younger men who are at high risk. PSA screening for prostate cancer is very sensitive tumoral marker, but it presents poor specificity. Risky, painful and costly biopsies need to integrate suspected occurrences of cancer to exclude the relevant number of false positives generated by PSA. Unfortunately, by biopsy a not insignificant number of false negative is present, delaying the cancer detection (prostate biopsy is a statistical sampling, and not a whole organ examination therefore then there are chances to miss the target).

The proposed bio-sensor goes in the direction to eliminate or strongly reduce this problem, offering a second degree of evaluation after PSA screening, sensitive and specific, able to examine the organ as a whole. Such a result is foreseen to enhance European competitiveness when transferred from research into commercially successful products in the fields of ultrasound and targeted UCAs technologies and participating industries have the necessary know-how and expertise to do this.

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