PROJECT REVIEW

"An Integrated Approach with Affinity-Based Biosensing (ABB) for Gene Doping Detection: A Pilot Study"

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Different analytical approaches can be foreseen for direct analysis and for the identification of a characteristic signature pattern following gene doping.

The pilot project will evaluate the proof of principle of Affinity Based Biosensors (ABB) integrated with bioinformatics and biomolecular approaches for gene doping detection.

Our challenge is to provide a total analytical process for the evaluation of the presence of the gene doping event. The heart of the project is a new multiscreening and real time bioanalytical protocol, based on an affinity sensing platform to be used both in direct and indirect based approaches for gene doping detection.
We believe affinity-based biosensors (ABBs), flanking conventional and profiling methodologies, can contribute to gene doping detection as fast, low cost and easy to use instrumental approach.

In this context, a flexible platform, consisting of a biochip coupled to a label free technology for simultaneous measurements in short time could represent an innovative approach for selectively detecting gene doping markers (direct approach) or secondary effects induced by gene transfer (indirect approach).
The feasibility of this project is assured by the high interdisciplinarity of the partners of the proponent team which will contribute with their specific competences to the definition of a total analytical process using bioanalytical, bioinformatics, biomolecular and immunological competences. The pilot project outcome, will be transferred to the Italian reference anti-doping laboratory (Federazione Italiana Medico Sportiva, Roma), accredited by WADA.

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Results and Conclusions

The present project aimed to develop an innovative analytical approach for delivering sampling and analytical protocol to be applied to gene doping detection, eventually setting up a database.

A new multiscreening and real time bioanalytical protocol, based on an affinity sensing platform for gene doping detection, was developed using an integrated multidisciplinary approaches based on bioanalytical, bioinformatics, biomolecular, and immunological competences.

In particular, we developed a bioinformatics supported study for the identification of suitable markers for gene doping tracing.

The initial purpose of the Gene Doping Detection Database (GDDDB) is to provide functionality for the design of primers on sequences that can be potentially used as Vectors during gene doping. Since it is currently the most commonly used gene therapy vector, the pilot study GDDDB contains Adenovirus sequences only. The database scheme is designed so that it can be interfaced by a biomart engine (www.biomart.org). Primer design is done using the Primer-BLAST web service provides by NCBI (www.ncbi.nlm.nih.gov/tools/primer-blast/), which is a web-based graphical interface to the Primer3 and BLAST algorithms. Here we describe the entity relationship diagram of the database, and then show how it is adjusted to a data warehouse scheme as required for use by the biomart engine. The GDDDB can be accessed and tested via the GDD portal at http://aragon.cl.cam.ac.uk/GDD/dbportal.html.

Furthermore a simple discrete Bayesian analysis is done to calculate the posterior probabilities of gene doping. These results are shown and are given for each probe used in the developed assay. The conditional posterior probabilities are also shown, depending on whether a high or low affinity has been observed from samples.

The project also developed an animal model (*in vivo* approach). The in vivo approach has first used transgenic mice to for the EGFP reporter gene to validate the molecular analysis of the marker in different tissue. Once the applicability of the developed method for the analysis of the selected marker has been proved in this first transgenic model, then a second model system based on the injection of the vector, containing the same reporter gene EGFP, in the tibialis anterior and of the femoral quadriceps muscles was developed. The sampling has been executed at different times after transfection and from different tissues: muscles, liver, spleen, kidney, lung, heart, right quadriceps (site of injection) and left quadriceps. Moreover body fluids (urine, blood, tears) have also been used to evaluate the presence of viral vector signature.

To trace the marker gene dedicated approaches have beel developed and applied to these animal models. The EGFP expression has also monitored in different tissue of transgenic mice.

In order to unambiguously detect the presence of recombinant vector, a protocol for construct-specific sequences was also developed. For this purpose new primers pairs were designed respectively on 3' promoter and 5' end of the EGFP sequences.

Finally different region of the EGFP marker was amplified for tracing the market in the mice after gene-doping event mimicking. The target sequences were found in all the sampled tissue.

Surface Plasmon Resonance imaging (SPRi)-based sensing for the detection of the gene-doping event was achieved. In particular Affinity Based biosensors (ABB) have been developed. Both for DNA target sequence detection (DNA sensing) and antibody detection in human serum (immunosensor) are reported.

Immobilization chemistries for molecular probe surface binding, analytical protocol were first optimized using standard solutions and further applied to complex samples (PCR mixture for DNA sensing –direct approach and serum for indirect approach).

The analytical platform (biochip) allows simultaneous and real-time detection of sequences belonging to the vector.

Publications

Conferences:

Oral presentations:

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2. S. Scarano, M. L. Ermini, M. M. Spiriti, P. Bogani, M. Buiatti, M. Mascini and M. Minunni, Affinity optical sensing based on Surface Plasmon Resonance imaging (SPR-i) in gene doping controls, Biosensors 2010, 20th Anniversary of World congress on Biosensors, 26-28 May 2010, Glasgow, UK (accepted)

Posters

1. M. Minunni, S. Scarano, C. Scuffi, M. Mascini, Surface Plasmon Resonance Imaging for Affinity-Based Biosensors, AISEM-Associazione Italiana Sensori e Microsistemi, XII Conferenza Annuale, Pavia, Italy 24-26 february **2009**
2. Scarano, S. Spiriti, M., Bogani, P., Buiatti, M., Mascini, M. and M. Minunni The challenge of gene doping detection: development of piezoelectric- and SPR imaging-based biosensors, 3rd European Conference on Chemistry for Life Sciences Frankfurt am Main, September 2 - 5, **2009**

Articles on Journals

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- S. Scarano, C. Scuffi, M. Mascini and M. Minunni Surface Plasmon Resonance Imaging (SPRi) –based sensing for the analysis of proteins in real matrices (in preparation)
- S. Scarano, C. Scuffi, M.L. Ermini, M. Mascini and M. Minunni Surfce Plasmon Resonance Imaging (SPRi)-based sensing: A new approach in signal sampling and managment (in preparation)
- S.Scarano, M.L. Ermini, C. Scuffi, P. Bogani, M.M. Spiriti, S. Lazzarano, P. Liò, A. Arcangeli, M. Buiatti and M. Minunni, **An integrated approach for gene doping detection: a pilot study** (in preparation)