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Sviluppo di un Dispositivo Diagnostico In Vitro Basato su Immuno-SERS per la Rilevazione Ultrasensibile e Rapida di Biomarcatori Tumorali

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Early diagnosis of tumors: studying cancer at the molecular level

Genomics

Technologies used include:

- DNA microarray
- DNA sequencing
- Multiplex PCR

These genomic technologies have proven effective in analysing molecular changes in tumours, allowing:

- DNA copy number assessment
- Mutation screening
- Gene expression profiling
- microRNA expression profiling

Proteomics and metabolomics

Technologies used include:

- Mass spectroscopy
- Liquid chromatography
- Protein microarrays
- Surface plasmon resonance
- NMR Spectroscopy

These technologies could provide effective tools for detecting tumour markers, proteins, hormones, enzymes, and peptides, which represent unequivocal signs of tumorigenesis or cancer progression

Identification and Detection of Tumour Biomarkers

Opportunities

Early detection of specific cancers

Types of Tumour Biomarkers:
PSA, Prostate Cancer
CA 125, Ovarian Cancer
CA 19-9, Pancreatic Cancer

Monitoring therapy response

Biomarkers can track cancer progression or response to treatment

Minimally invasive approach

Identifying biomarkers in biological fluids (e.g., blood, plasma, serum) may reduce the need for solid tissue biopsy

Limitations

Lack of strong correlation with cancer risk

Difficulties to conclusively link biomarker presence with actual cancer development
Potential for false positives or false negatives

Undefined clinical significance

Many biomarkers do not yet have a clear clinical role

Technical limitations of detection methods

ELISA, Colorimetric Assays, Electrochemiluminescence
Highlight sensitivity issues.
Surface Plasmon Resonance, Mass Spectrometry
Highlight complexity and cost challenges

New methods that are ultrasensitive, simple, and easily transferable to clinical use are needed.

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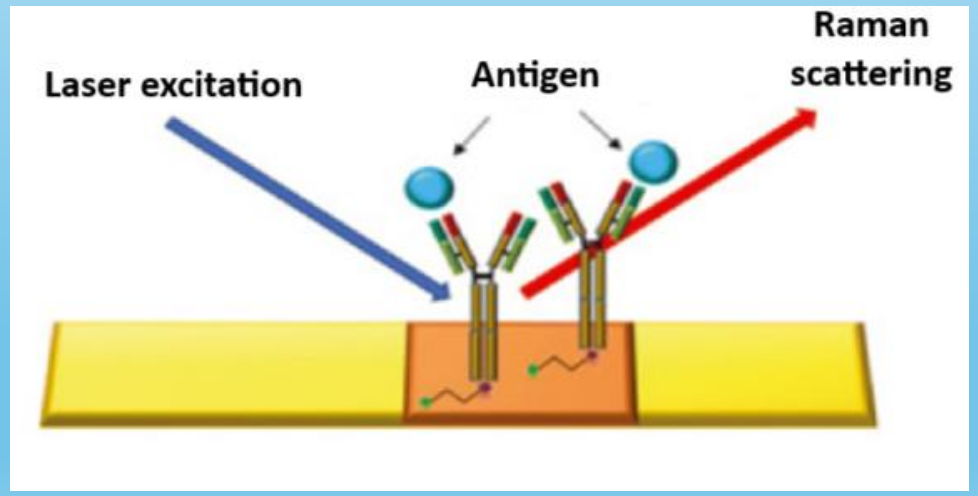
Surface Enhanced Raman Scattering (SERS) for Ultrasensitive Detection of Tumour Markers

SERS couples the **power of Raman spectroscopy in molecule identification** with the **drastic improvement of the signal** mediated by plasmonic materials (*e.g.* gold, silver).

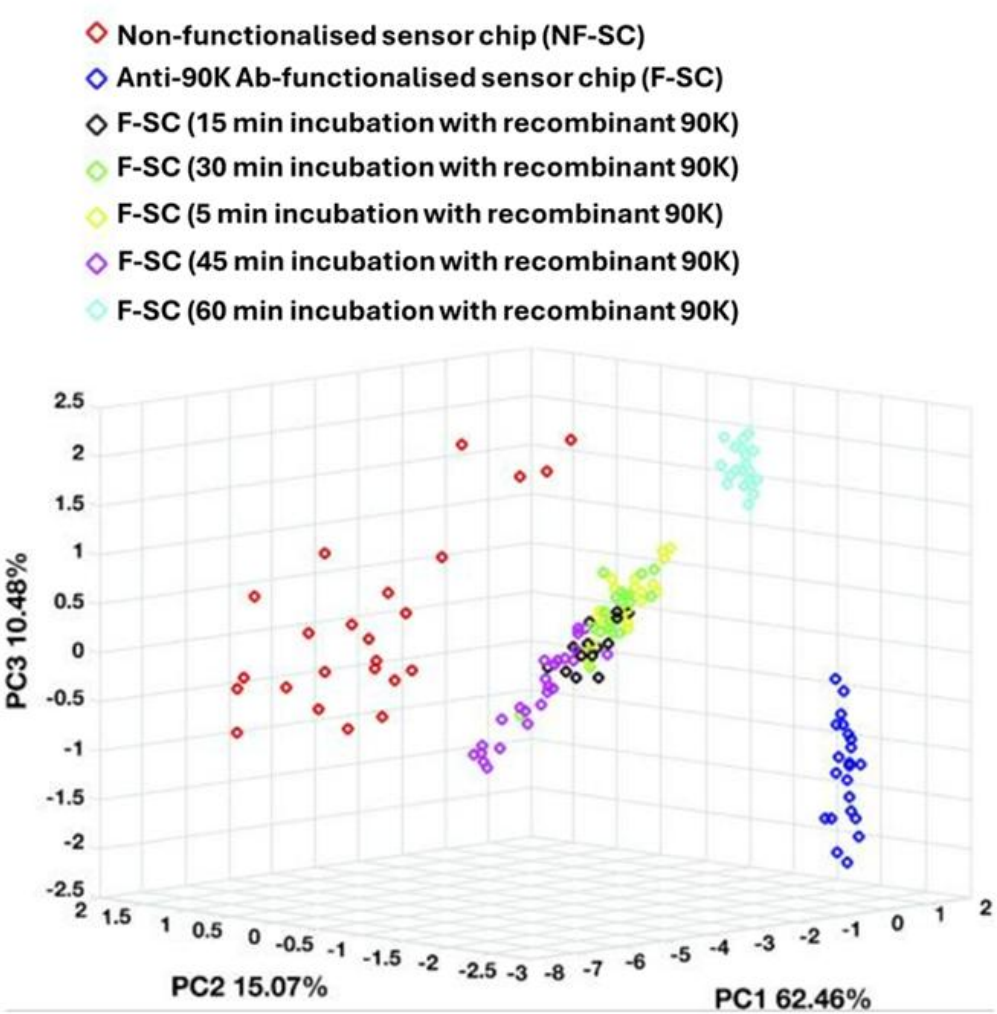


We employed a direct immuno-SERS detection method for the ultrasensitive detection of Gal3-BP

Galectin-3 binding protein (90K) was employed as a tumour marker model to assess the system's effectiveness in ultra-sensitive biomarker detection within complex biological fluids, such as serum.

Validation of the SERS detection system

A recombinant 90K protein was used as a standard to evaluate antigen/antibody interaction.



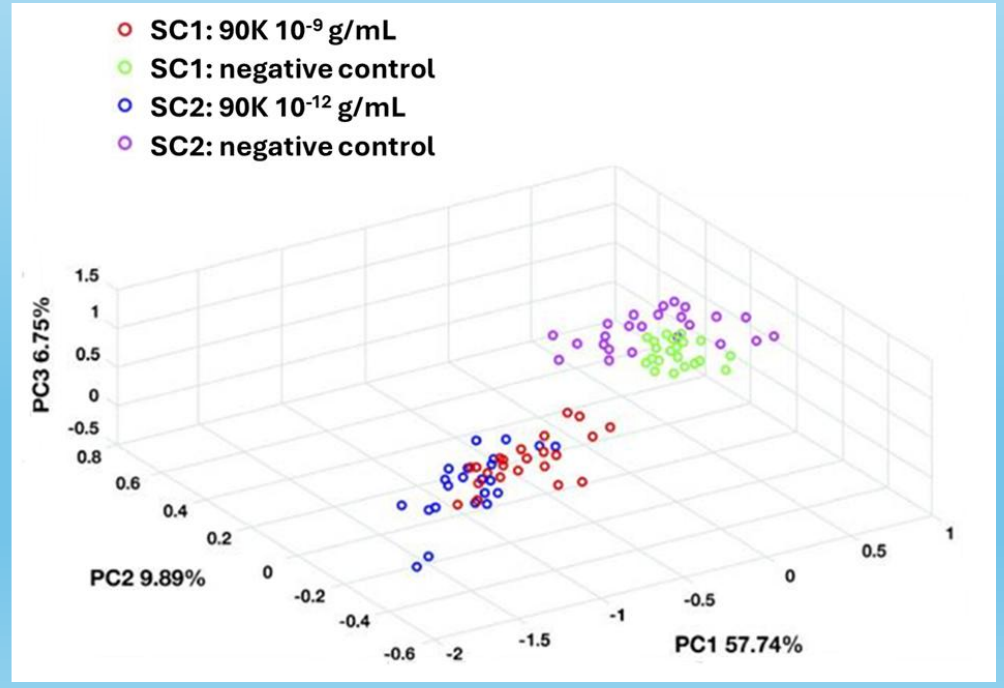
Sensor chips were incubated with known concentrations of recombinant 90K, starting from 0.5 µg/ml—a concentration reliably detectable by ELISA.

The 90K/antibody interaction was monitored over 60 minutes to determine the optimal incubation time. **Principal Component Analysis (PCA)** of the Raman spectra revealed a clear distinction between sensor chips before and after exposure to 90K, with **measurable spectral separation observed as early as 5 minutes**.

These findings demonstrate **the rapid responsiveness of the detection system**, capable of identifying antigen–antibody interactions within minutes.

Validating the detection system with serum from cancer patients

Following preliminary quantification of the 90K antigen concentration via ELISA, serum samples were diluted by factors of 10^6 and 10^9. The diluted samples were then incubated on the functionalized sensor chips for 30 minutes to allow antibody–antigen binding.



PCA of Raman spectra

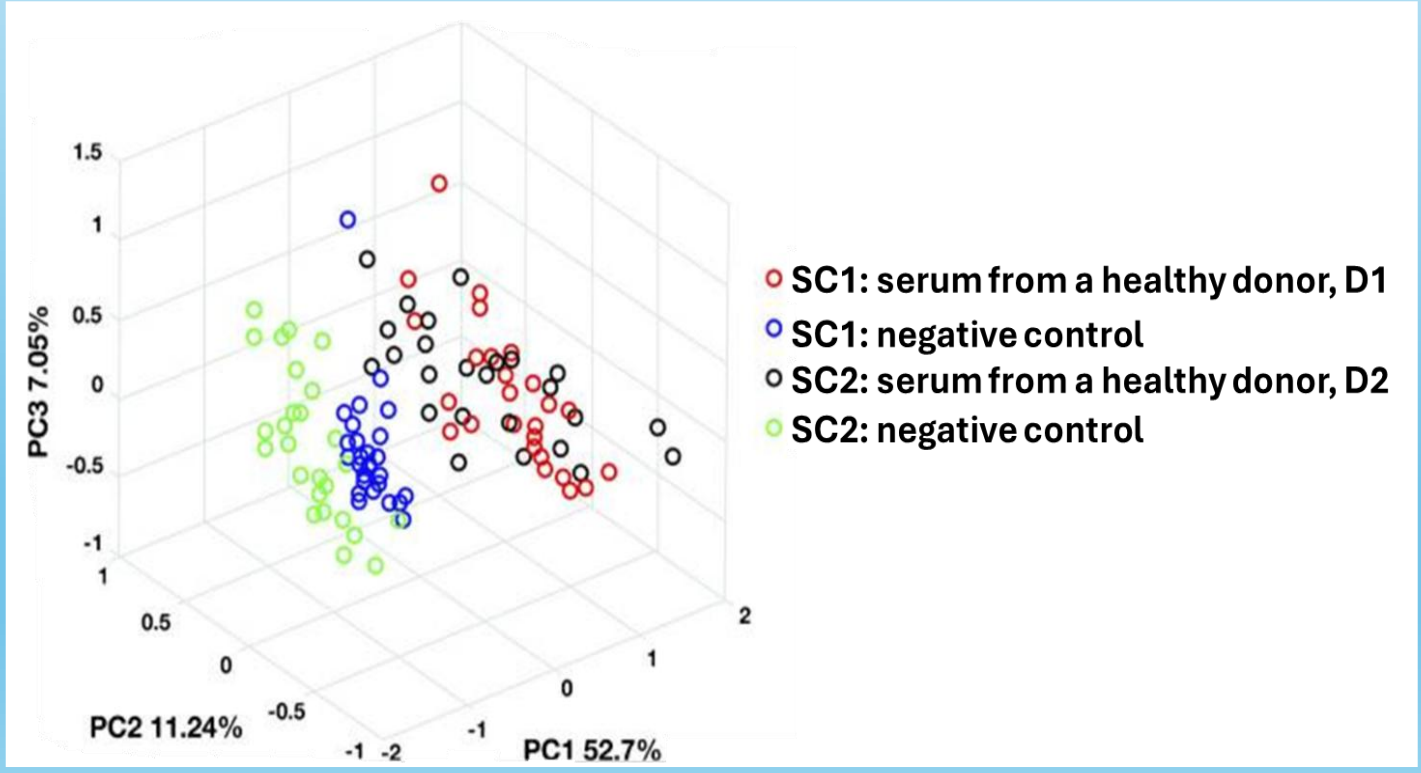
Subsequent SERS analysis enabled detection and characterization of the 90K antigen.

PCA data revealed a **clear separation** between the antibody-functionalized sensor chips before and after incubation with serum samples, detectable even at a 10^9 dilution of cancer serum (corresponding to a **90K concentration of approximately 10^{-12} g/ml**).

These results demonstrate the system's effectiveness in detecting the 90K antigen at picogram levels.

Validating the detection system with serum from healthy patients

As for oncologic patients, serum samples from healthy patients were diluted by factors of 10^6 (D1) and 10^9 (D2). The diluted samples were then incubated on the functionalized sensor chips for 30 minutes.



PCA of Raman spectra

PCA of **healthy serum samples**—lacking the 90K tumor marker but still containing all other serum components present in oncologic patients—**showed no distinct clustering between pre- and post-incubation sensor chips.** This indicates that other serum constituents do not produce a detectable Raman signal under these conditions, thereby **confirming the specificity of the detection system for the 90K antigen**.

Obtained results:

Sensitivity: the SERS-based detection system is effective for the **ultrasensitive detection** of the 90K antigen in serum from oncologic patients (**limit of detection 10^{-12} g/ml**).

Specificity: the system is not affected by interferences from other serum components.

Simplicity: the direct immuno-SERS technique enables fast and simple analyses.

Versatility: the functionalization procedure can be effectively applied to various antibodies; multiplexed antibody-functionalized SERS substrates can be developed to study biomarker panels.

Development of an innovative integrated IVD automated medical device for the early diagnosis of cancer

- Based on the developed direct immuno-SERS methodology, which allows a quick and ultrasensitive detection of **specific biomarkers in complex biological matrices,** we are **prototyping an integrated medical device with superior predictive power, compared to other diagnostic techniques**.

Characteristics of the automated device:

1. **Development of an integrated HW/SW system for analyzing and classifying biological data and bioinformatics tools. Intelligent System**: automation in interpreting biological data.

2. **Development of an Expert System with Agent capabilities for detecting biomarkers. Self-learning**: the system improves its predictive power over time.

3. **Development of a user interface** that allows the operator to easily control the device, acquire, process, and manage biological data and patient information. **Reduction of reliance on experts** (medical researchers) for data interpretation.

Conclusions and perspectives

- The integrated automatic device would significantly advance medical diagnostics, enhancing the accuracy and speed of oncological diagnoses.

- The device's features would make it suitable for **point-of-care testing (POCT)** or allowing it to be performed in outpatient settings or "spoke" locations of clinical facilities, not necessarily requiring high-level diagnostic excellence.

- Altogether, these characteristics could pave the way for effective and accessible proximity medicine, ultimately improving patient care and outcomes by making advanced diagnostics more readily available to those in need.

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