PLATFORM FOR MULTIFUNCTIONAL PARTICLES IN BIOSENSING APPLICATIONS

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In recent years, there has been significant development of hydrogel-based technologies for a range of applications including diagnostics, drug delivery, and tissue engineering. Hydrogels are versatile materials due to their hydrophilic, biofriendly, and highly tunable nature, making them applicable in the detection of various biomolecules. The hydrogel-based platform is composed of multifunctional particles which possess multiple components and properties editable to fulfil the requirements of the applications. Core-shell particles in which the core and the shell act distinct roles, as well as particles incorporated or conjugated with other functional species, find extensive applications. The chemical flexibility of hydrogel microparticles and microgels allows the embedding of several building blocks into their structure during the synthesis. Particles decorated with anchoring groups can be exploited to immobilize probes of diverse natures such as antibodies, enzymes, or oligonucleotide strands. Particles active for the detection of a target or more than one biomarker can be obtained. The high biocompatibility and antifouling properties of hydrogel make possible the employing of the microgels-based platform in biosensing and diagnostic fields for the detection of more biomarkers. Core-shell microgels have been opportunely synthesised and functionalized with oligonucleotide probes for their employing in nucleic acid assays. The microgels-based platform enables the target detection based on a fluorescence readout by recovering a signal with a proportional intensity to the target amount. Higher sensitivity and lower limit of the detection than assays have reached with the same probes not conjugated to microgels. This result is due to the confinement of probes in the small volume of particles producing an enhancement of fluorescent signal.

Multiplex assays allow the simultaneous measurement of target analytes from the same sample, decreasing the time and cost. Microgels with different spectrally encoding are obtained by embedding diverse fluorophore dyes in different ratios into the core and the shell. Assays have been performed to detect more than one biomarker into the serum sample. Fluorescence emission by probe highlighted the presence of targets whose identities were revealed by reading out each microgel barcode. The multifunctional particles-based platform represents a modular technique that can be generalized for any direct detection applied to a wide spectrum of biomedical applications.

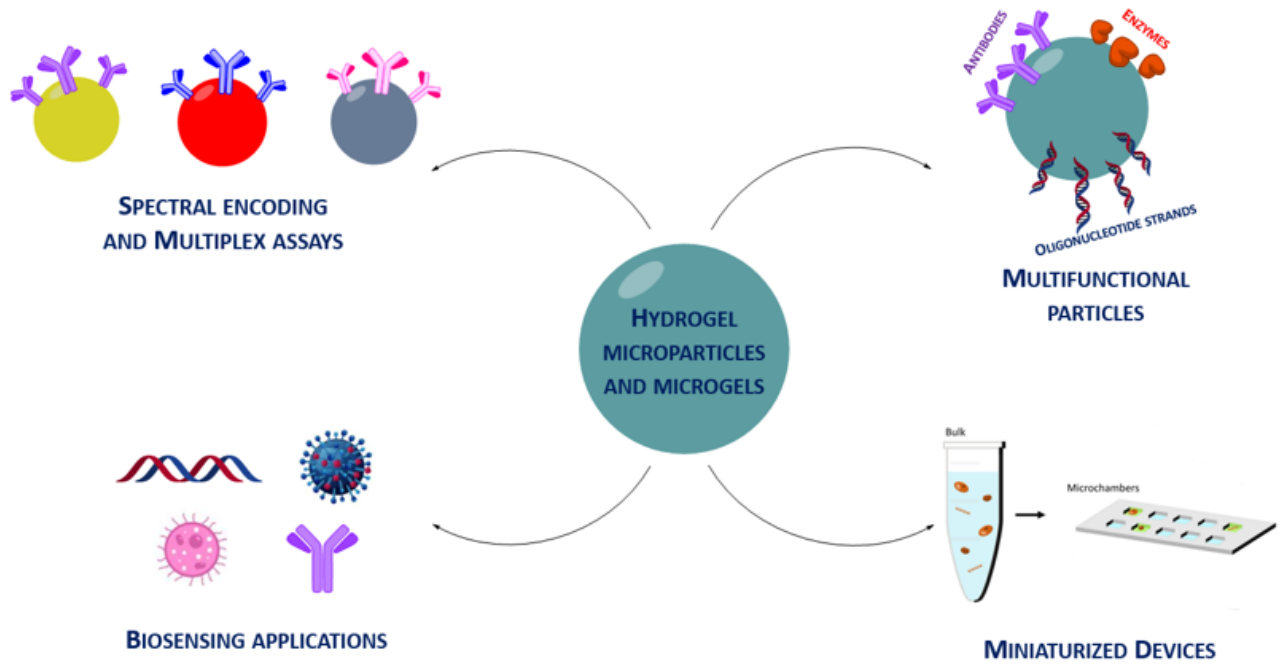
My PhD project aims to widen the applications of the hydrogel-based platform towards new goals in the biosensing field to have innovative bioassays. The hydrogel-based platform will be applied to develop an ultrasensitive and innovative immunoassay for the detection of new biomarkers such as proteins and small molecules. Nowadays, immunoassays are the most spread analytical procedures used to measure proteins in the diagnostic field. Analytes and disease biomarkers are usually present in fluid and serum at subfemtomolar concentrations. For this reason, methods featured by both sensitive target detection and broad analytical ranges must be designed. Innovative formats will be explored combining particles and probes of diverse nature to reach the most appropriate assay. Multifunctional particles will be designed and developed with different materials and combined with probes such as antibodies, aptamer, enzymes, and proteins. The flexibility of the hydrogels-based platform will be used to modulate parameters affecting the sensitivity and the limit of detection of the assay. By doping particles with fluorescent entities in different concentration ratios, unique codes can be generated. Many barcodes exploitable in multiplex assays can be created to widen the spectral encoding already used. The goal will be the simultaneous measurement of several target analytes from the same sample.

The potential of the microgels-based platform will give the possibility to overcome limited abilities of standard techniques already in use. Approaches based on single-entity detection and miniaturized structures are increasingly spreading to overcome the limits linked to the sensitivity. Single-Molecule Array (SiMoA) is an approach that makes use of arrays of femtoliter-sized reaction wells that can detect single molecules. By restricting sample volume to a single entity, a high local concentration is generated easily to detect thanks to the signal amplification in the confined volume. The flexibility of the particles-based platform allows decreasing the number of particles involved in the assay. The application of the single-entity method and the use of microfluidic devices can lead to the development of bioassay characterized by ultrahigh sensitivity. The successive experiments will concern the quantification of antibodies coated onto microgels surface.

In the second part of the work, I will proceed to design a specific format for the assay. A possibility could be a competitive assay in which functionalized microgels and a competitor are involved. The BSA protein linked to the target T and a fluorophore dye could be chosen for the competition with the target antigen. The saturation of the binding sites of antibodies onto particles will be performed by using the competitor. The interaction with the free target T will allow the displacement of the competitor. The decrease of the signal will be directly proportional to the amount of the target T. The recovering of the signal can be performed by using both confocal laser scanning microscope and the spectrofluorometer. The confocal laser scanning microscope will give the possibility to improve the assay because a smaller number of particles can be visualized and their fluorescence signal quantified.

Microgels and hydrogel microparticles will be designed to embed organic dyes, nanoparticles as quantum dots and upconversion nanoparticles into their structures in different ratios. Thus, a large number of barcodes exploitable in multiplex assays can be created to widen the spectral encoding already used. By doping particles with multiple entities in different concentration ratios, unique codes with an increase in encoding capacity can be obtained. The barcode of the encoding system should be robust and stable with high resistance to environmental conditions such as pH, temperature, and buffer concentrations. It must be wide and easy to detect. The goal will be the simultaneous measurement of different target analytes from the same sample, decreasing the time and cost. Multiplex assays will be tried to increase the number of targets detectable and to widen the encoding system.

In the last part of the work, the flexibility of the hydrogels-based platform will be used to modulate parameters affecting the sensitivity and the limit of detection of the assay. The flexibility of the hydrogel-based platform allowed to modulate the number of particles involved in bioassay to try improving the number of biomarkers detected. The application of the single-entity method to the hydrogel-based platform and its miniaturization in microfluidic devices can lead to the development of bioassay characterized by ultrahigh sensitivity. The idea is to decrease further the number of particles manipulated in the assay and in turn, to decrease the examined part of the sample under test. The use of miniaturized device combined with the three-dimensional hydrophilic polymer networks directs towards the more and more sensitive assay and to push these technologies as a point of care device.



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