Abstract

The here proposed work is aimed to introduce new functionalities on a microgravimetric sensor based on a suspended microchannel resonator (SMR). In particular, a novel label-free sensing approach is envisioned, conceiving a high-capturing substrate (porous beads), used as mass-carrier, with 3D laser machined SMR devices. In order to do that, a deep knowledge of their peculiar functionalities as distinctive systems is required.

The SMRs has shown great potential in the biosensing field. In particular, the design proposed by *Manalis et. al* has been extensively applied to the study of eukaryotic cells, ranging from the monitoring of single cell growth to the determination of the therapeutic susceptibility. The main drawback of the actual SMR platforms is represented by the fabrication process, which generally requires expensive, time consuming and complex processing techniques. Moreover, only few examples of label-free biomolecular assays based on these sensors have been presented in the literature. These approaches generally exploit the selective functionalization of the inner walls of the suspended channel, in order to promote the immobilization of specific biomolecular capturing probes (i.e. ssDNA, antibody or other macromolecules). Unfortunately, this method shows some limitations mainly due to the inhomogeneity of the functionalization of the channel walls and the control of the fluid dynamic aspects to promote the binding kinetics between probe and target molecules.

This work shows for the first time an innovative, rapid and monolithic fabrication process to get totally transparent SMRs. The SMRs have been developed entirely in glass with a 3D microfabrication approach that simplifies and speeds up the standard fabrication process. Starting from a microscope glass slide, a femtosecond laser micromachining technique directly defines, within a single step, both the suspended bridge resonator and the embedded microfluidic channel that are subsequently released by a KOH etching. The mechanical characterization of these resonating bridges showed that the mass resolution and sensitivity in air environment are comparable to those reported for state-of-art SMRs, while they perform better than commercial microcapillary glass resonators.

In order to develop a label free bioassay with the glass SMRs, a new approach was investigated: the use of a mesoporous bead as high binding capturing vector was proposed. The idea comes from the possibility to exploit the same characterization concept used for the study of the single cell, flushing the beads inside the channel and monitoring the mass variation due to the interaction between probe and target molecules. Using this approach, the limitation due to the functionalization inside the channel is avoided, thanks to the possibility to directly add specific functionalities during the beads synthesis. At the same time, the optimization of the detection assay can be preliminary conducted using well-known biomolecular strategies. In this perspective, the thesis work was mainly devoted to the development of a bioassay compatible with the integration into the SMR platform and in particular it was mainly focused on the optimization of a DNA-DNA hybridization assay using the mesoporous silica beads as capturing substrate.

The work started with the optimization of a singular strategy of synthesis to obtain micron-size porous beads compatible with the inner channel dimension. A co-condensation method, in acidic environment, was preferred to other synthesis strategies. In fact, using this approach it was possible to obtain, with high efficiency, mesoporous beads with different chemical functionalities already embedded into the porous framework. After a careful physical-chemical characterization, the MPTMS (3-mercaptopropyltrimethoxisilane) modified beads were chosen as preferential material for the immobilization of the ssDNA probe. The non-specific signals, deriving from undesired interactions between the molecules involved in the assay, represent a crucial aspect for the future integration of the assay into the SMR platform. In fact, this phenomenon will affect the evaluation of the beads mass variation generating false positive results. For this reason, the optimization of the bioassay was carried out searching the best configuration for the non-specific signal suppression. The optimization of the molecular recognition allows to obtain a relatively high limit of detection (LOD), which ranges between 11 and 3 pM, thus being comparable with well-know biomolecular tests such as the enzyme linked immunosorbent assay (ELISA). The dynamic range and the LOD relative to the bead-based assay could be in principle further improved with the integration of this protocol with the quantification of the target amount directly with the SMR platform, using a label-free approach.