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Implementation of a PDMS Microdevice for the Improved Purification of Circulating MicroRNAs

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Abstract : The relevance of circulating miRNAs as non-invasive biomarkers for several pathologies is nowadays undoubtedly clear, as they have been found to have both diagnostic and prognostic value able to add fundamental information to patients' clinical picture. The availability of these data, however, relies on a time-consuming process spanning from the sample collection and processing to the data analysis. In light of this, strategies which are able to ease this procedure are in high demand and considerable effort have been made in developing Lab-on-a-chip (LOC) devices able to speed up and standardise the bench work. In this context, a very promising polydimethylsiloxane (PDMS)-based microdevice which integrates the processing of the biological sample, i.e. purification of extracellular miRNAs, and reverse transcription was previously developed in our lab. In this study, we aimed at the improvement of the miRNA extraction performances of this micro device by increasing the ability of its surface to absorb extracellular miRNAs from biological samples. For this purpose, we focused on the modulation of two properties of the material: roughness and charge. PDMS surface roughness was modulated by casting with several templates (terminated with silicon oxide coated by a thin anti-adhesion aluminum layer), followed by a panel of curing conditions. Atomic force microscopy (AFM) was employed to estimate changes at the nanometric scale. To introduce modifications in surface charge we functionalized PDMS with different mixes of positively charged 3-aminopropyltrimethoxysilanes (APTMS) and neutral poly(ethylene glycol) silane (PEG). The surface chemical composition was characterized by X-ray photoelectron spectroscopy (XPS) and the number of exposed primary amines was quantified with the reagent sulfosuccinimidyl-4-o-(4,4-dimethoxytrityl) butyrate (s-SDTB). As our final end point, the adsorption rate of all these different conditions was assessed by fluorescence microscopy by incubating a synthetic fluorescently-labeled miRNA. Our preliminary analysis identified casting on thermally grown silicon oxide, followed by a curing step at 85°C for 1 hour, as the most efficient technique to obtain a PDMS surface roughness in the nanometric scaleable to trap miRNA. In addition, functionalisation with 0.1% APTMS and 0.9% PEG was found to be a necessary step to significantly increase the amount of microRNA adsorbed on the surface, therefore, available for further steps as on-chip reverse transcription. These findings show a substantial improvement in the extraction efficiency of our PDMS microdevice, ultimately leading to an important step forward in the development of an innovative, easy-to-use and integrated system for the direct purification of less abundant circulating microRNAs.

Keywords : circulating miRNAs, diagnostics, Lab-on-a-chip, polydimethylsiloxane (PDMS)
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