



## **Trypsin Immobilization for Use on Digital Microfluidic Devices**

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Droplet-based microfluidics is an emerging field in which nanoliter-sized droplets can be manipulated by changes in local electric fields. Specifically, the wettability of a hydrophobic dielectric layer changes in response to potentials applied to electrodes patterned below the surface. This phenomenon can be exploited to move, split, and merge droplets of polar liquids, thus enabling reactions, phase transfers, and biological assays on the micro-scale. This lab-on-chip technique consumes far less reagents than traditional methods, reaction times are typically shorter (as diffusion distances are smaller), and the device is suitable for automation. One promising application for this technology is for proteomics. To prepare proteins of interest for identification by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI), several steps must be completed including proteolytic digestion. Immobilized trypsin was examined as an alternative to a trypsin digest in solution because this expensive enzyme is reusable if immobilized to a solid support within the device. Trypsin was immobilized in both a porous silicon dioxide sol-gel and tethered to magnetite silica-iron-oxide nanoparticles. BSA-FITC immobilized in sol-gel did not wash away, even when subjected to heavy washing, which suggests large proteins are retained well within the silica matrix. Tryptic digestion rates and efficiency were measured for digestions of bovine insulin B-chain as a target peptide. MALDI mass spectra of the digestion product of immobilized trypsin showed efficient and complete proteolysis. These immobilization techniques are efficient and show promise for use in peptide digestion as well other enzyme-catalyzed reactions on digital microfluidic devices.