

Plasma polymer surfaces for high-throughput microfluidic proteomic analysis

G.J.S.Fowler¹, A.M.Pereira², B.O'Sullivan², G.Mishra¹, P.C.Wright², S.L.McArthur¹

¹ Dept. of Engineering Materials, The Kroto Research Institute, University of Sheffield, UK

² Dept. of Chemical and Process Engineering, University of Sheffield, UK

INTRODUCTION: Proteomics, the identification and quantification of the protein component of parallel biological samples, has at its core the procedures of protein extraction, protein separation, proteolytic cleavage and mass analysis. In order to move on from 2D-gel-based procedures it is necessary to design high-throughput microfluidic gel-free devices. In this study the use of plasma polymerisation allows the introduction of both physical and chemical characteristics to microchannels. We demonstrate a proteolytic microreactor as well as microfluidic devices that allow the isolation of subclasses of peptides from a peptide mixture.

METHODS: Plasma polymerisation, absorbance and fluorescence spectroscopy, X-ray photoelectron spectroscopy, LC-MS/MS mass spectrometry, ELISA

RESULTS & DISCUSSION:

Plasma polymers have been used for the covalent immobilisation of various enzymes and as a platform for protein/peptide immobilising transition metal ions. Plasma polymers are used because they can be introduced onto virtually any substrate to produce coatings of a control thickness and composition.

Trypsin has been covalently immobilised to plasma polymerised poly(acrylic acid) and its activity tested with L-BAPA, epicocconone-labelled proteins (LavaDigest) and mass spectrometry.

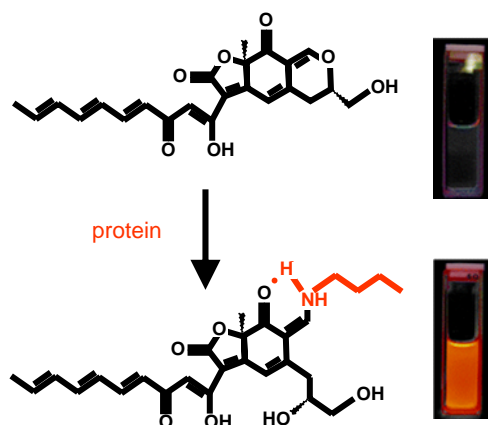


Fig 1: Fluorescent complexes of proteins combined with tryptic peptide identification by MS are used to test effectiveness of proteolytic surfaces.

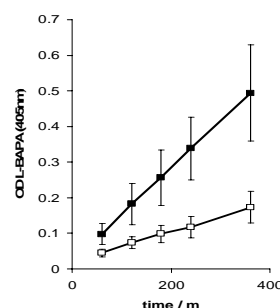


Fig 2. Activity of trypsin EDC/NHS-preimmobilised onto polyacrylic acid plasma polymer surfaces under static conditions as assessed using colorimetric agent L-BAPA. (Trypsin concentration in PBS solution during immobilisation; ■ 0.5mg/ml, □ 0.1mg/ml).

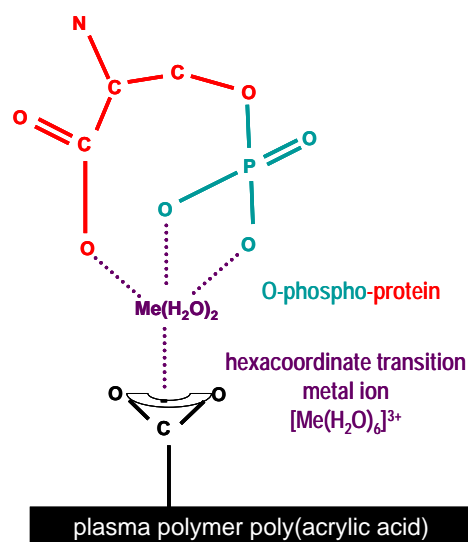


Fig 3: Plasma polymer poly(acrylic acid) used as a platform for immobilised metal ion affinity chromatography (IMAC) The phosphorylated protein O-phospho-L-serine-BSA (see above) has been isolated from solution using transition metal-treated plasma polymer surfaces, and the process assessed using anti-phospho-antibody ELISA and spectroscopy.

CONCLUSIONS: We have demonstrated that plasma polymers can be used both to immobilise proteins and peptide-selective metal ions. In this way we can produce microfluidic components for high-throughput gel-free proteomic analysis.