HYBRID CERAMIC-POLYMER MICROFLUIDIC CHIPS FOR BIOMOLECULE SEPARATIONS

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ABSTRACT

A commercial hybrid material, ORMOCOMP®, is introduced to the fabrication of separation microchips and applied to bioanalytical applications. With theoretical plates approaching 10^6/m and detection limit of proteins at low picogram level (10^{-12} g, by laser induced fluorescence), our results suggest that ORMOCOMP® holds record-breaking performance in microchip electrophoresis with respect to both separation efficiency and detection sensitivity over other polymer-like materials.

KEYWORDS: ceramic-polymers, electrophoresis, fluorescence, bioanalysis

INTRODUCTION

During the past years, various polymer-like materials have been proposed as structural material for microfluidic separation chips thanks to their low cost fabrication. Organically modified ceramics (ORMOCER®s) are a new group of commercial materials that allow fast and simple patterning by UV-lithography or UV-embossing [1-3]. The physico-chemical properties of ORMOCER®s can be tailored toward different applications, e.g. for optical devices [1] or antistatic and antiadhesive coatings [2]. In this paper, a commercial hybrid material, ORMOCOMP®, is for the first time introduced to the fabrication of enclosed separation microchips and successfully applied to bioanalytical applications.

EXPERIMENTAL

Three-layered, fully ORMOCOMP® separation microchips were fabricated by UV-lithography and bonding techniques [3], and their performance was examined in the analysis of a variety of biomolecular samples by means of microchip electrophoresis (MCE). All compounds were detected as their fluorescein isothiocyanate (FITC) conjugates by laser induced fluorescence (LIF) microscopy at 488 nm (ex). All amino acids and peptides were labeled in house with a two-fold excess of FITC in 50 mM $NaHCO_3$ (pH 9.0) with 20% acetone. Protein tryptic digests (29:1) were incubated at +37 °C for approximately 18 h and were purified by C18 ZipTip® solid-phase extraction followed by FITC-labeling prior analysis. FITC-bovine serum albumin (BSA) was a commercial conjugate. Sample introduction was performed in pinched injection mode (15-20 s) through a double-T crossing. The separation methods were optimized with respect to electric field strength and separation buffer composition.

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RESULTS AND DISCUSSION

Highly efficient MCE separations with plate numbers as high as 5.5-8.3×10^5/m (i.e., 2.5-3.8×10^4 per channel length) were easily achieved for all amino acids and peptides within 60 seconds on an ORMOCOMP® chip (Fig. 1). Record-breaking performance was also obtained in protein analysis without any surface modification or surface active ingredients applied to suppress nonspecific adsorption effects. Peak asymmetry and peak widths of FITC-bovine serum albumin (BSA) were perfectly comparable to those on a commercial Borofloat® glass chip suggesting negligible interactions between the protein and the ORMOCOMP® surface (Fig. 2a). Moreover, run-to-run repeatabilities (n=4-6) for the migration time of FITC-BSA were consistent with the repeatabilities of the electroosmotic flow (EOF) on both materials and also consistent between the two materials (Table 1). Moreover, ORMOCOMP®'s low absorptivity at near UV and visible wavelengths allowed highly sensitive LIF detection with limit of detection at low picogram (1.4×10^{-12} g) level for FITC-BSA and with good linearity over the range of 10-1000 μg/mL (Fig. 2b).

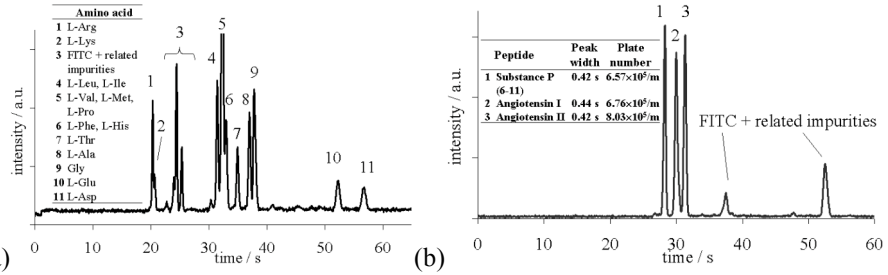

Figure 1. (a) Separation of 50 μM FITC-labeled amino acids on an ORMOCOMP® microchannel (30 μm×20 μm, w×h). The separation buffer was 40 mM sodium borate (pH 10.0), electric field strength 800 V/cm, and detection distance 4.5 cm. (b) Separation of 200 μM FITC-labeled peptides on an ORMOCOMP® microchannel (50 μm×20 μm, w×h). The separation buffer was 20 mM sodium borate (pH 10.0), electric field strength 700 V/cm, and detection distance 4.0 cm.

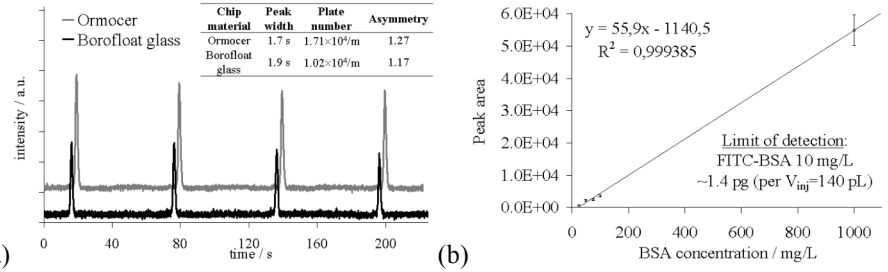

Figure 2. (a) Repeated injections of 1 mg/mL FITC-BSA on ORMOCOMP® and Borofloat® microchannels (both 50 μm×20 μm, w×h). Separation conditions were as per Fig. 1b. (b) Calibration curve of FITC-BSA between 0.01 and 1.0 μg/mL.

Table 1. Comparison of run-to-run and chip-to-chip repeatabilities on ORMOCOMP® and Borofloat® glass microchips.

Micrchip material	*run-to-run(n=4-6)*		*chip-to-chip (n=3)*	
	EOF / $\times 10^{-8}$ $m^2/(V\times s)$	**FITC-BSA migr. time / sec**	**EOF/$\times 10^{-8}$ $m^2/(V\times s)$**	**FITC-BSA migr. time / sec**
Ormocomp®	3.3±0.3 (7.7%)	19.3±0.2 (0.9%)	2.7±0.5 (19%)	29.2±7.2 (26%)
Borofloat®	4.5 ± 0.1 (1.7%)	16.1±0.2 (1.1%)	5.4±0.9 (17%)	20.5±4.5 (22%)

The high resolving power of the ORMOCOMP® chips was also proven by the analysis of tryptic digests of β-lactoglobuline and bovine serum albumin (BSA), which both showed over 20 peaks for their characteristic peptide fingerprints (Fig. 3). When compared to commercial Borofloat® glass microchips, similar resolving power for the peptide sample (Fig. 1b), for example, was only obtained with much longer separation path lengths. Even so, plate numbers for the peptide separation in Borofloat® glass microchannels under optimized conditions were somewhat lower (3.8-5.3×10^5/m) than those in ORMOCOMP® channels (6.6-8.0×10^5/m).

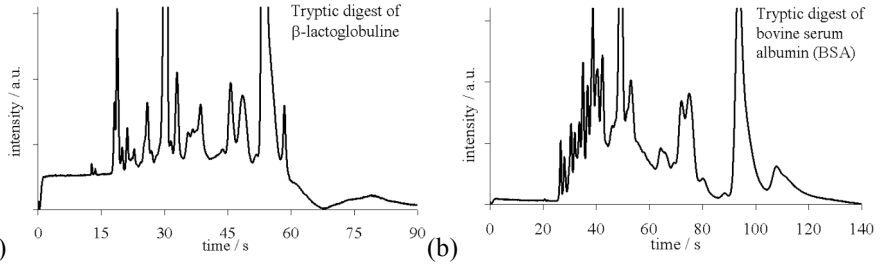

Figure 3. Separation of FITC-labeled tryptic digest of (a) β-lactoglobuline and (b) BSA in ORMOCOMP® microchannel of 50 μm×28 μm (w×h). The electric field strength was (a) 700 V/cm or (b) 500 V/cm, other conditions as per Fig. 1b.

CONCLUSIONS

To our knowledge, this is the first report on microfluidic separations performed using enclosed, fully ORMOCOMP® microchannels. Our results suggest that ORMOCOMP® holds record-breaking performance with respect to separation efficiency and detection sensitivity over other polymer-like materials.

ACKNOWLEDGEMENTS

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