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Solid-material-based coupling efficiency analyzed with time-of-flight secondary ion mass spectrometry

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ABSTRACT

The coupling behavior of a microparticle embedded amino acid active-ester into a Poly(ethylene glycol)methacrylate-film, synthesized onto a silicon wafer by a grafting from approach, is characterized using dynamic time-of-flight secondary ion mass spectrometry (ToF-SIMS) to analyze the 3d distribution of the amino acids in the polymer film. Besides standard solid phase peptide synthesis, employing solubilized amino acids in a solvent, we used solid polymer microparticles, incorporating the amino acids. These microparticles were especially designed for a new technique to produce high-density combinatorial peptide microarrays: upon heating, the particles become viscous, which releases the embedded amino acids to diffuse and couple to the surface. In the scope of the development of this new particle-based application, ToF-SIMS is used to analyze a complex chemically modified polymer surface layer. Due to depth profile measurements, it is possible to investigate the particle-based coupling reaction not only on the surface, but also into the depth of the PEGMA film.

1. Introduction

In 1992 Ronald Frank developed the SPOT-Technique, an automated approach for a full combinatorial peptide synthesis in the array format on a membrane support [1,2]. This procedure is based on the concept of the solid-phase peptide synthesis (SPPS), which was invented by Bruce Merrifield in 1963 [3]. Today, the SPOT-Technique (see Fig. 1a) or variants thereof are commercially applied by companies like e.g. INTAVIS Bioanalytical Instruments AG [4], JPT Peptide Technologies GmbH [5], and others [6–8]. The combinatorial diversity arising from the 20 different amino acids is the major challenge for *in situ* peptide array synthesis. Meanwhile several research groups have investigated and reported novel strategies for the production of high-density peptide arrays [9]. Lithographic approaches, combining photomasks with photo-labile protecting groups [10,11] or more advanced technologies using digitally controlled patterned illumination to generate acids for deprotection by photolytic reactions [12] represent applied synthesis pathways. In 2007 and 2008, two straightforward xerographic methods to produce high-density combinatorial peptide microarrays were presented [13,14]. These techniques use polymer-based, amino acid loaded microparticles, which are addressed to a functionalized, Poly(ethylene glycol) methacrylate-(PEGMA) coated support [15,16] by electromagnetic fields (see Fig. 1b). The microparticles' hull consists of a commercially available polymer that was developed for toner particles in xerographic applications [14]. Microparticles are produced by dissolving one type of amino acid derivative and the polymer matrix material in a solvent, which is then evaporated in a spray dryer. The resulting polymer microparticles embed the amino acid derivatives. This material forms a brittle solid at room temperature but turns viscous at elevated temperatures. The viscous polymer efficiently confines the material to very small reaction sites, but at the same time frees the embedded amino acid derivatives to diffuse and couple to the surface (see Fig. 1c). Custom-made xerography toner-batches, one for each of the 20 amino acids, were developed and characterized to produce peptide microarrays. Towards the

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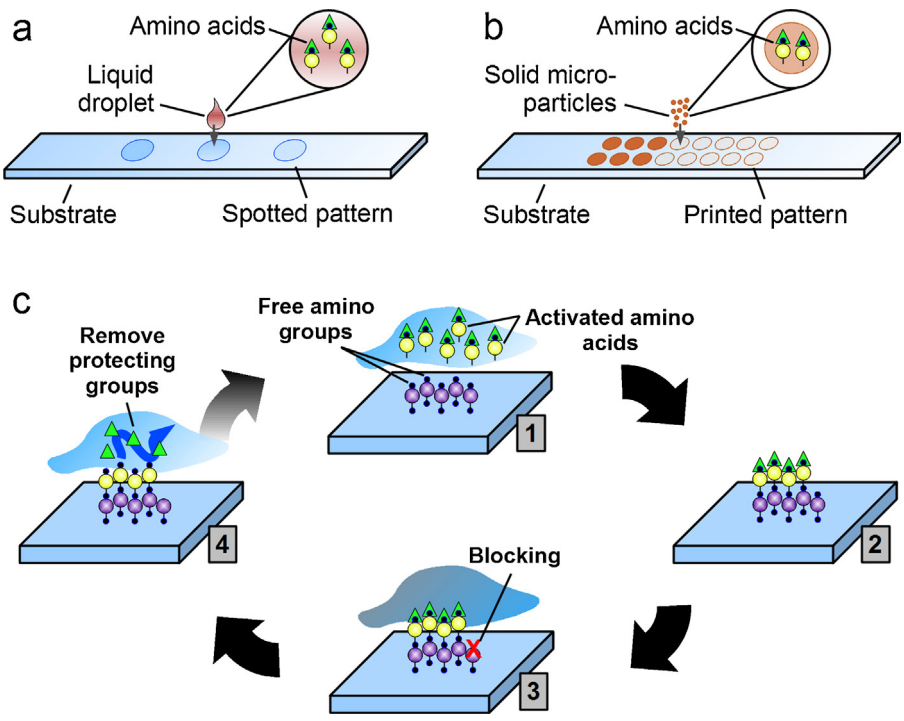

Fig. 1. Combinatorial synthesis of peptides. Amino acids are deposited on a surface with (a) the SPOT technology or (b) the particle-based technology. Activated amino acids in a solvent directly couple to the surface upon deposition, whereas solid microparticles have to be heated, to induce the coupling reaction. (c) The coupling reaction is performed in four steps: (1) deposition and (2) coupling of amino acids to amino groups, (3) blocking of unreacted amino groups and, (4) removing the amino-terminal protecting groups.

Table 1
Important aspects for SIMS molecular depth profiling.

Parameters and considerations	Applied system	Alternative approaches	Refs.
Sample			
Sputtering yield and damage creation	Poly(ethylene glycol)methacrylate exhibits a suitable balance of erosion and degradation of molecular structures due to sputter ion bombardment	Poly(lactide) and poly (methyl methacrylates) are suitable In contrast, Poly(styrene) undergoes cross-linking under irradiation and is intractable, see refs	[25], [27]
Low initial roughness	Spin coating layer, see text		
Homogeneity	Phase separated polymers would result in preferential sputtering and roughening of the sample during erosion		[28]
Instrumentation			
Sputter beam	Cs^+, 500 eV, no persistent molecular signals C_{60}^+, 20 keV, trityl signals detectable, see text	Small projectiles: Ar^+, Xe^+, O_2^+, Cs^+ rarely applicable: Rapid decay of molecular fragments Clusters: SF_5^+, Ar_x^+ provide often superior results	[29]
Analysis beam	Bi_3^+ field emission source, 25 keV, approx. 1 ns pulse width, high spectral resolution, and approx. 5 μm lateral resolution. Dose lower than 5% of sputtering dose to avoid extensive subsurface damage	C_{60}^+, single beam depth profiling with lower lateral resolution (20 μm), or long Bi pulses for improved lateral resolution (<500 nm)	[30]
Depth calibration	Performed by off-line profilometry at craters of different sputter beam fluences	In-line AFM analysis without air contact of the sample	[31]
Sample temperature	Room temperature	Cryogenic temperatures if necessary (possible cross linking can be reduced, providing constant erosion rates and good depth resolution)	[32]
Sample rotation	Fixed sample position allows for 3D imaging. Analysis beam and sputter beam from opposite directions, both 45° towards sample	Zalar rotation (not synchronized with data recording) for depth profiling of laterally homogeneous samples only. Improved depth resolution and sputtering yield was observed. Synchronized, 90° stepwise rotation	[30], [32], [33]