Optics and Lasers in Engineering 76 (2016) 57–63

Contents lists available at ScienceDirect

Optics and Lasers in Engineering

journal homepage: www.elsevier.com/locate/optlaseng

Probing droplets with biological colloidal suspensions on smart surfaces by synchrotron radiation micro- and nano-beams

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ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form
10 February 2015
Accepted 3 March 2015
Available online 24 March 2015

Keywords:
Biological colloids
Digital microfluidics
Micro-/nano X-ray diffraction
MicroFTIR
Synchrotron radiation

ABSTRACT

Droplets with colloidal biological suspensions evaporating on substrates with defined wetting properties generate confined environments for initiating aggregation and self-assembly processes. We describe smart micro- and nanostructured surfaces, optimized for probing single droplets and residues by synchrotron radiation micro- and nanobeam diffraction techniques. Applications are presented for Ac-IVD and β-amyloid (1–42) peptides capable of forming cross-β sheet structures. Complementary synchrotron radiation FTIR microspectroscopy addresses secondary structure formation. The high synchrotron radiation source brilliance enables fast raster-scan experiments.

1. Introduction

The evaporation of droplets of colloidal biological suspensions results in various types of patterns depending on the nature of surface interactions [1]. Indeed, coffee-ring type residues are formed on wetting surfaces [2]. More complex patterns are observed on superhydrophobic surfaces (SHS) ranging from spherical to collapsed coffee-ring type residues depending on the solute concentration [3]. The formation of these "confined environments" is due to convective flow and diffusion mediated mass transport to the droplet interface resulting in the formation of gelated layers [4] which are at the origin of aggregation and self-assembly processes. Microscopic evidence for such processes can be obtained by probing droplets or residues by X-ray micro- and nanobeam scattering techniques at high-brilliance synchrotron radiation (SR) sources [3]. Wide-angle X-ray scattering and small-angle X-ray scattering techniques (summarized here as micro X-ray diffraction: μXRD) in combination with raster-scan probing reveal structural features from atomic to macroscopic scales [3,5–7]. Spectroscopy probes with raster-scan capability, such as Fourier transform infrared microspectroscopy (μFTIR), can provide complementary information.

This text will review methodological advances in fabricating structured substrates with tailored wetting capabilities, optimized for μXRD raster-scan probing. The use of such substrates will be demonstrated for selected short peptides capable of forming nanofibrillar cross-β sheet phases [8]. We will also discuss μFTIR experiments addressing secondary structures formed during peptide self-assembly. All experiments reported were performed at the European Synchrotron Radiation Facility (ESRF), a state-of-the-art 3rd generation SR source.

2. Methods

2.1. Substrate technologies

Structured substrates for μXRD probing of droplets should -ideally- have the following properties: (i) low X-ray absorption, (ii) low X-ray background scattering, (iii) none or weak

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http://dx.doi.org/10.1016/j.optlaseng.2015.03.004
0143-8166/

shadowing-effects, (iv) tailored wetting behaviour and (v) predetermined aggregation or self-assembly points.

Pillared Si-SHSs (Fig. 1A), based on Si-wafers of ~500 μm thickness, are a well-established technology [9] but pose problems as supports for weakly X-ray scattering biological specimens due to high X-ray absorption (e.g. ~82% at $\lambda \sim 1$ Å [10]). The signal/noise ratio of X-ray signals is, however, high thanks to the low X-ray scattering background of single-crystalline silicon. As alternative way, a more X-ray transparent substrate such as ~500 μm thick polymethylmethacrylate (PMMA) sheets can be chosen to fabricate PMMA-SHSs [11]. The strong diffuse X-ray scattering background from the PMMA sheet requires, however, precise background subtraction techniques [11]. Although experimental considerations usually prevail, reducing the number of steps in producing a structured substrate can be a valuable economic argument. Indeed, creating a SHS from PMMA by surface roughening via plasma etching (Fig. 1C; left) requires fewer fabrication steps than a pillared PMMA-SHS [11]. It is also interesting noting that by reducing the nanofibrillar PMMA density and in the absence of a Teflon layer, the surface wetting properties can be tailored from superhydrophobic to superhydrophilic (Fig. 1C; right) [12,13]. A thin PMMA film can also be spin-coated and structured on highly X-ray or IR transparent substrates such as Si_3N_4 membranes, [13] allowing reducing absorption and diffuse X-ray scattering.

Depending on the position of the X-ray beam on a droplet, the edge of a SHS will more or less shadow the μXRD pattern for a SR-beam aligned parallel to the surface ("horizontal geometry": HG; see Figs. 2 and 3). Shadowing problems can be an issue when probing a droplet or residue close to the surface. Highly X-ray transparent, thin Si_3N_4 substrates with SU-8 pillars are better suited for HG-mode probing provided that the surrounding Si-frame is also thin (Fig. 1E) [10]. Moreover, it has the intrinsic advantage that the residue can be easily detached from the substrate and posed to a thin capillary tip (Fig. 4A) [16]. This is, however, not possible for fragile morphologies such as nanofilaments which have to be probed on the substrate in NG-mode. In order to reduce and locally avoid absorption in NG, one can use a pillared Si-SHS based on a thin silicon substrate (< 50 μm) with etched holes (Fig. 1D) [15]. The variation of X-ray absorption across the substrate requires, however, elaborate data treatment for absorption corrections which can be avoided in practice by using a SHS based on a thin Si_3N_4 membrane with SU-8 pillars (Fig. 1E) [10]. The radial pillar-gradient shown in Fig. 1E generates in addition a pinning centre which allows keeping the droplet at a constant position during evaporation. An alternative is provided by fabricating a cone in a forest of silicon pillars via ion-beam milling (Fig. 1E) [9]. Pillar gradients and cones are of particular interest for concentrating ultradilute solution droplets at predetermined points for probing experiments [9,17,18].

In summary, pillared SHSs based on Si_3N_4 membranes and SU-8 pillars [10] provide a significant advantage for probing weakly scattering organic or biological materials with SR scattering techniques. PMMA-SHSs based on PMMA thin films with nanofibrillar surface roughness have a considerable potential, in particular for FTIR applications [13]. Pillared Si-SHSs based on standard Si-wafers remain of interest for stronger scattering materials in view of a well-established nanofabrication technology. Pillared Si-SHSs with holes are used for TEM applications [9] but are not optimal for X-ray scattering in view of local absorption variations.

2.2. Synchrotron radiation scattering techniques

High brilliance SR is produced in a so-called storage ring by electron bunches moving close to the speed of light through periodic magnetic devices (e.g. undulators) [19]. The main elements of a μXRD beamline are shown in Fig. 2. SR emitted in a narrow cone from an undulator is monochromated ($\Delta E/E \sim 10^{-4}$) by a double Si-crystal to -typically- $E \sim 13$ keV ($\lambda \sim 0.95$ Å) and focused at sample position by refractive, reflective or diffractive optical elements.

μXRD experiments reported below were performed at the ESRF-ID13 beamline which uses currently refractive lenses made of Be or Si providing routinely beam sizes from a few μm down to ~100 nm with a flux up to $\sim 9 \times 10^{11}$ photons/s in an about 2×3 μm^2 focal spot at ~13 keV [7,20–22]. Experiments are generally performed in air although the control of humidity would allow modulating the droplet evaporation rate by up to about factor 7 [3]. Droplets are generally probed in HG-mode but a grazing-incidence geometry is

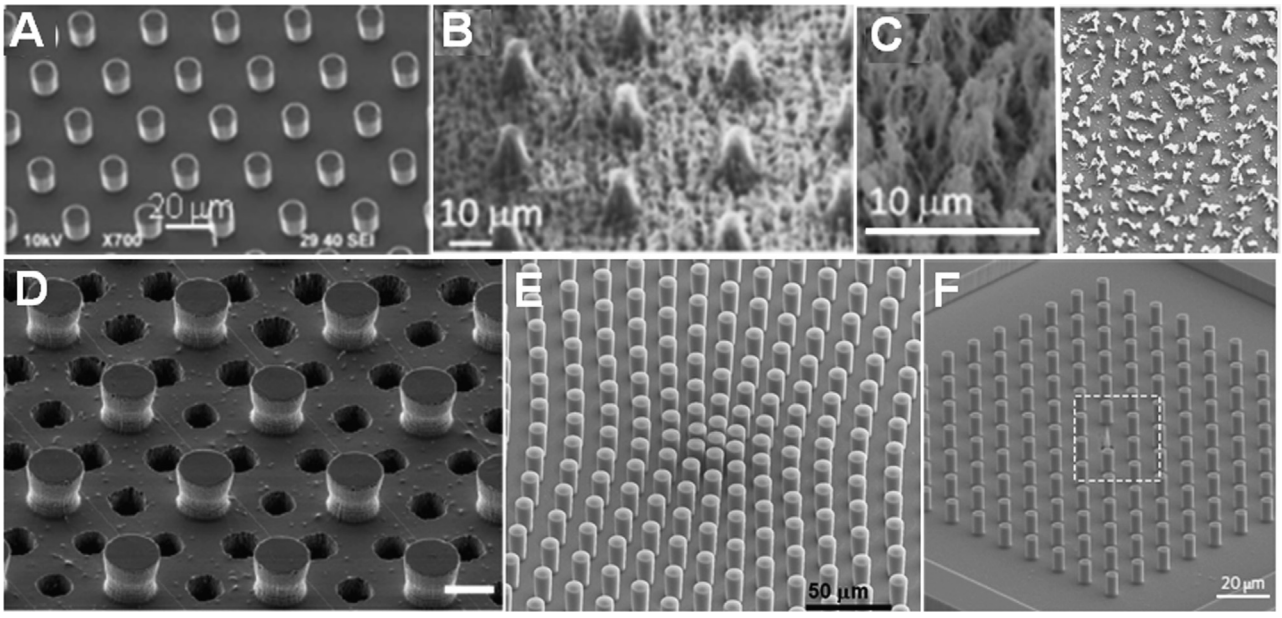

Fig. 1. SEM images for selected microfabricated surfaces (A): Si-SHS with a forest of Si-pillars. (B): PMMA-SHS with micropillars showing nanofibrillar roughness. (Adapted from: [11]) (C): Left: PMMA-SHS with a high-density nanofibrillar PMMA surface. (Adapted from: [14]); Right: Superhydrophilic Si_3N_4 substrate with a low-density nanofibrillar PMMA surface (same scale). (D): Pillared Si-SHS with 6 μm diameter, etched holes developed for transmission electron microscopy. (Adapted from: [9]) (E): SHS based on a Si_3N_4 membrane and a gradient of SU-8 pillars. (Adapted from: [10]) (F): Pillared Si-SHS with central nanocone. (Adapted from: [15]) The pillar-gradient (E) and the nanocone provide an attraction potential for an evaporating droplet.