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Polymer surface properties control the function of heavy meromyosin in dynamic nanodevices

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ABSTRACT

The actin-myosin system, responsible for muscle contraction, is also the force-generating element in dynamic nanodevices operating with surface-immobilized motor proteins. These devices require materials that are amenable to micro- and nano-fabrication, but also preserve the bioactivity of molecular motors. The complexity of the protein-surface systems is greatly amplified by those of the polymer-fluid interface; and of the structure and function of molecular motors, making the study of these interactions critical to the success of molecular motor-based nanodevices. We measured the density of the adsorbed motor protein (heavy meromyosin, HMM) using quartz crystal microbalance; and motor bioactivity with ATPase assay, on a set of model surfaces, i.e., nitrocellulose, polystyrene, poly(methyl methacrylate), and poly(butyl methacrylate), poly(tert-butyl methacrylate). A higher hydrophobicity of the adsorbing material translates in a higher total number of HMM molecules per unit area, but also in a lower uptake of water, and a lower ratio of active per total HMM molecules per unit area. We also measured the motility characteristics of actin filaments on the model surfaces, i.e., velocity, smoothness and deflection of movement, determined via in vitro motility assays. The filament velocities were found to be controlled by the relative number of active HMM per total motors, rather than their absolute surface density. The study allowed the formulation of the general engineering principles for the selection of polymeric materials for the manufacturing of dynamic nanodevices using protein molecular motors.

1. Introduction

Mechanical work in biological nanosystems is performed by a variety of force-generating protein motors, such as myosins, kinesins and dyneins (Spudich, 2011; Vale, 2003; Veigel and Schmidt, 2011), the former being responsible for muscle contraction (A.F. Huxley and Niedergerke, 1954; H. Huxley and Hanson, 1954). In the "gliding geometry" motility assay developed in the late 1980s, whole myosin molecules (Kron and Spudich, 1986; Uyeda et al., 1991), or the part of the mechano-enzyme containing its working arms, e.g., heavy meromyosin (HMM) (Uyeda et al., 1991), or even the end of an arm, i.e., the S1 unit (Toyoshima et al., 1987; Uyeda et al., 1991), are adsorbed on a surface. Provided that the upper solution contains sufficient adenosine triphosphate (ATP), the fluorescently-labelled actin filaments will be propelled by the surface-bound motors, sliding randomly on the surface, thus allowing the easy observation and quantification of motility characteristics using simple optical fluorescence microscopy set-ups and imaging software.

While the "gliding geometry" motility assay has been used extensively in fundamental studies of molecular motor function (Holzbaur and Goldman, 2010), from an applications perspective, their planar architecture and the ability of motor proteins to transport nano-scale cargo at speeds that are orders of magnitude higher than those associated with molecular diffusion (Nitta and Hess, 2005), are very attractive features for dynamic nanodevices (Bakewell and Nicolau, 2007; Fulga et al., 2009; Kinbara and Aida, 2005) Consequently, proof-of-concept motor-powered nanodevices have been proposed for biosensing (Agarwal et al., 2009; Martinez-Neira et al., 2005; Van Zalinge et al., 2012), biodiagnostics (Fischer et al., 2009; Korten et al., 2010), transport at nano- (Bull et al., 2005), and micro-scale (Limberis and Stewart, 2000), microfluidic pumping (Bull et al., 2005) and recently

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biocomputation (Nicolau et al., 2016).

Because the manufacturing of these devices must use materials that are both suitable for micro/nanofabrication, and also preserve the motor bioactivity, various materials have been assessed, e.g., methacrylate polymers (Nicolau et al., 1999; Riveline et al., 1998; Suzuki et al., 1997), polyurethane (Clemmens et al., 2003a), plasma polymerised poly(ethylene oxide) (Clemmens et al., 2003b), polyelectrolytes (Jaber et al., 2003), commercial photoresists (Bunk et al., 2003a, 2003b; Clemmens et al., 2004; Hiratsuka et al., 2001; Moorjani et al., 2003), and silane-functionalized surfaces (Bunk et al., 2005; Sundberg et al., 2003). Despite this rather large amount of empirical information, as well as several studies focused on the fundamentals of the motor protein-surface interactions (Albet-Torres et al., 2007a; Katira et al., 2007, 2009; Van Zalinge et al., 2012), there are still many uncertainties regarding the impact of surfaces on motor function, in particular regarding polymers, which are the preferred materials for inexpensive devices, due to the coupled complexities of the polymer and the protein systems.

To this end, to progress on the selection of materials for future dynamic nanodevices using actin-myosin system, we studied the relationship between the physico-chemical properties polymeric surfaces, in particular their hydrophobicity and polymer network structure, on one side; and the surface density of molecular motors and the preservation of their motility, on the other.

2. Materials and methods

2.1. Polymer surface coating

Superclean nitrocellulose (NC) was purchased from Ernest F. Fullam, Inc. (Latham, NY). Polystyrene (PS), poly(methyl methacrylate) (PMMA), poly(butyl methacrylate) (PBMA), poly(*tert*-butyl methacrylate) (PtBMA) and hexamethyldisilazane (HMDS) were purchased from Aldrich Chemicals. The selection of polymeric surfaces aims to reach a reasonably large range of properties related to motility assays and related devices: (i) nitrocellulose is the standard polymer for motility assays, but unfit for the fabrication of devices due to its flammability; (ii) polystyrene is the material of choice for the plastic utensils in molecular biology and biochemistry, but is rarely used for motility assays; (iii) PMMA is the material of choice for polymer-based microfluidics devices, and has been also used with good results for motility assays; (iv) it would be useful to compare PMMA with other more hydrophobic acrylates, but with very different properties related to water uptake, i.e., high, and low Tg, for PtBuMA and PBMA, respectively. Finally, while silane polymers, e.g., poly(di methyl siloxane), PDMS, are used indeed for microfluidics devices, the release of mono-/oligo-mers is extremely toxic for motility assays.

The chemical structures for all model surfaces are presented in Fig. 1.

Glass coverslips were cleaned by sonication in 70% ethanol, dried in a stream of N_2, primed with HMDS, spin-coated with one of the polymer solutions, i.e., NC (1% w-v in amyl acetate), PS (2.5% w-v in propylene glycol monomethyl ether acetate, PGMEA), PMMA (2% w-v PGMEA), PBMA (1% w-v toluene), or PtBMA (2% w-v in PGMEA) at 3600 rpm, then soft-baked at 85 °C for three hours. The concentrations of solvents in the polymer solutions have been optimised to obtain a viscosity that leads to a smooth film during the spin coating.

The hydrophobicities of the polymer-coated surfaces were determined by contact-angle measurements using deionized water ($R > 18.2\ M\Omega$) and Krüss contact-anglemeter (DSA10Mk2). The reported values are averages of ten different readings for each surface.

The measurement of the viscoelastic properties of the polymers in thin films used an advanced commercial quartz microbalance (QCM) system (QCM-Z500, from KSV Instruments). This system allowed the measurement of the impedance spectrum, thus providing both the frequency and the bandwidth, addressing up to 11th harmonics. The measurements have been performed sequentially, in a step-wise manner, i.e., first on the bare dry polymer surfaces, then on surfaces interfaced with the buffer solution. A complete description of the QCM equipment, measurement protocols and associated theoretical background is presented in the Supplementary information section.

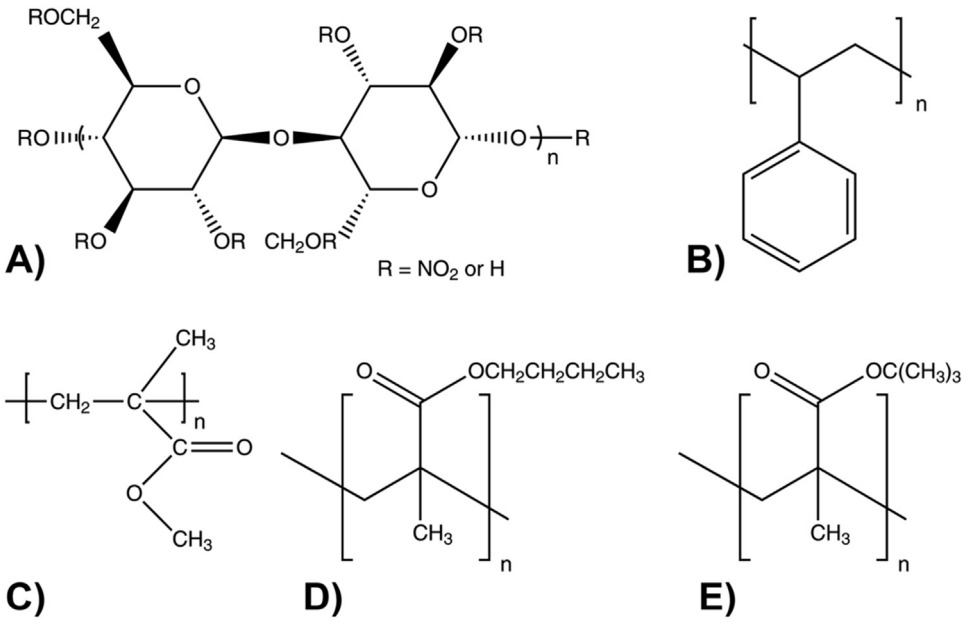

Fig. 1. Chemical structures of surfaces and polymers tested for in vitro actomyosin motility support. A: nitrocellulose (NC); B: poly(styrene) (PS); C: poly(methyl methacrylate) (PMMA); D: poly(butyl methacrylate) (PBMA); G: poly(tert-butyl methacrylate) (PtBMA).

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