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Contemporaneous cell spreading and phagocytosis: Magneto-resistive real-time monitoring of membrane competing processes

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ABSTRACT

Adhesion and spreading of cells strongly depend on the properties of the underlying surface, which has significant consequences in long-term cell behavior adaption. This relationship is important for the understanding of both biological functions and their bioactivity in disease-related applications. Employing our magnetic lab-on-a-chip system, we present magnetoresistive-based real-time and label-free detection of cellular phagocytosis behavior during their spreading process on particle-immobilized sensor surfaces. Cell spreading experiments carried out on particle-free and particle-modified surfaces reveal a delay in spreading rate after an elapsed time of about 2.2 h for particle-modified surfaces due to contemporaneous cell membrane loss by particle phagocytosis. Our associated magnetoresistive measurements show a high uptake rate at early stages of cell spreading, which decreases steadily until it reaches saturation after an average elapsed time of about 100 min. The corresponding cellular average uptake rate during the entire cell spreading process accounts for three particles per minute. This result represents a four times higher phagocytosis efficiency compared to uptake experiments carried out for confluently grown cells, in which case cell spreading is already finished and, thus, excluded. Furthermore, other dynamic cell-surface interactions at nano-scale level such as cell migration or the dynamics of cell attachment and detachment are also addressable by our magnetic lab-on-a-chip approach.

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1. Introduction

Cell-surface interactions such as cell spreading and phagocytosis represent important aspects in biology and are of special interest for biomedical applications. Adherent cells like fibroblasts continually probe their environment, and they need to attach to and spread on an underlying surface in order to perform numerous biological functions such as embryogenesis, maintenance of tissue structure, proliferation, differentiation, wound healing, metastasis or uptake of infectious agents as part of the immune response (Bardsley and Aplin, 1983; Cretel et al., 2010; Mrksich, 2000). A crucial parameter for immediate and long-term cell behavior is the surface characteristic of the adhesive substrate, comprising not only biomolecular and chemical features, but also physical properties such as stiffness, roughness and topography (Cavalcanti-Adam et al., 2007; Cretel et al., 2010). Thus, by designing nearly arbitrary surface characteristics using micrometer/ nanometer patterning techniques and biochemistry, the biocompatibility of biomaterials used for implants or rapid wound healing applications can be improved by targeted tailoring of the cell-substrate interface bioactivity (Anselme, 2000; Jones, 2001; Pierres et al., 2003).

The cellular decision making process to either spread on a surface or to remain rounded determines in most cases the fate of the cell, i.e. survival or initiation of programmed cell death (apoptosis). During the functional phases of cell spreading, thin lamellipodial protrusions creep onto the substrate surface, which lead to a shape-transformation from an initially spherical to a finally disk-shaped state with a steady increase of the surface-to-volume ratio (Döbereiner et al., 2004, 2005). Common for processes such as cell spreading and phagocytosis is their need for additional cell plasma membrane, the supply of which is limited. In fact, the entire cell spreading process can be considered as the attempt of a cell to internalize a particle that is too large for phagocytosis. Indeed, experiments on phagocytosis of particles by granulocytes showed that both processes obey similar characteristics (Evans et al., 1993; Herant et al., 2006; Stewart et al., 1989).

Apart from conventional optical end-point detection methods, other optical techniques such as internal reflection microscopy (IRM) or total internal reflection fluorescence microscopy (TRIFM) are employed to follow the process of cell spreading with high spatial and temporal resolution (Burmeister et al., 1998; Cretel

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et al., 2010; Ryzhkov et al., 2010). In addition to the visualized cell/surface contact area, also the separation distance between the cell and the substrate surface can be quantified.

In this study we investigate the phagocytic behavior of human fibroblast cells during their spreading process on particle-immobilized sensor surfaces. Special focus is put on the susceptibility of cells during their adhesion process to the cell-membrane competitive mechanism of phagocytosis. To monitor the influence of particle uptake on the spreading characteristics in real-time, we employ our previously introduced magnetic approach based on magnetoresistive (GMR) sensors, magnetic particles and microfluidics (Shoshi et al., 2012). Research on real-time monitoring of cell-surface interactions at nano-scale level is of high importance in cellular biophysics, material science and the development of future biomaterials for biomedical applications (Gardel and Schwarz, 2010).

2. Magnetoresistive detection principle

The concept of magnetic real-time monitoring of cell phagocytosis during their spreading processes is based on measuring changes of the local magnetic stray field of pre-immobilized superparamagnetic particles (beads) within embedded magnetoresistive sensors. These stray field variations are induced by distance changes of the beads relative to the sensor during cell-bead interaction. The approach is sketched in Fig. 1. Initially, beads are immobilized onto various sensors of the biochip surface. The bead-induced response of the underlying sensor depends on the magnetic moment, the number of immobilized beads as well as the mean vertical separation distance $r(t)$ to the sensor layer. When cells attach to and spread on the chip surface, they start to internalize the beads, which results in an increase of their mean vertical distance (Fig. 1(b)). With the increasing distance, the stray field strength of a bead at a point within the sensor region decreases approximately by r^{-3}, which results in a lower sensor signal. As long as all other parameters are fixed, the sensor output decreases proportionally to the time dependent progress of cell spreading and phagocytosis, which allows continuous monitoring in real-time. Besides the general advantages of our magnetic approach (Shoshi et al., 2012), another unique feature is the capability of measuring the average post-phagocytosis bead-to-sensor separation distance within living cells. In view of drug delivery applications, this information could be used to study in addition to phagocytosis also possible subsequent exocytosis behavior. By tailoring the surface bio-chemistry and/or physical characteristics of the magnetic drug carriers, this methodology might be used to analyze and tune the drug dwell time in cancerous cells/tissue, thus improving their efficiency.

3. Materials and methods

The most relevant constituent parts of our magnetic lab-on-a-chip (MAGLab) setup as well as a detailed description regarding the sensor-chip fabrication and characteristics, bead properties and micromagnetic simulations can be found in Shoshi et al. (2012). Therefore, only a brief summary is given here.

3.1. MAGLab setup

Two decoupled pairs of Helmholtz (HH) coils generate homogeneous magnetic fields parallel (in-plane, max. ± 23 kA/m) and perpendicular (out-of-plane, max. ± 36 kA/m) to the chip plane. These fields are required for sensor characterization and magnetizing the beads during the sensor read-out process. In the center of both HH-coils a chip holder is positioned which is also equipped with an external temperature control. The fluidical and electrical connections are established by a connector lid. In addition, we use a long-range microscope with a CCD camera for on-chip optical observations. The magnetic fields as well as the read-out of the sensors are computer controlled (LabView™, www.ni.com).

3.2. Sensor-chip characteristics

A continuous stack of ten $Ni_{80}Fe_{20}$/Cu-double layers in the second antiferromagnetic coupling maximum (AFCM) is sputter-deposited on a 20 mm × 20 mm silicon substrate and patterned into meander-shaped sensors. Each chip consists of 16 sensors of four different sizes arranged in two identical rows, representing reference and magnetic particle detection sensors (ref- and biosensors). The resistance of each sensor is about 6–8 kΩ and its GMR amplitude is around 12%. All sensors are protected by a 230 nm thick Si_3N_4 passivation layer from interactions with cells and/or fluids. The surface of the passivation layer is functionalized by a 2% APTES solution to ensure cell growth to the chip-surface and immobilization of surface-modified beads. For all cell experiments, a fluidic channel system made of cross-linked polydimethylsiloxane (PDMS) is mounted on the chip which enables direct access solely to the biosensors for magnetic particle immobilization and cell incubation. After surface functionalization and PDMS mounting, the chip is assembled into the chip holder of the MAGLab setup.

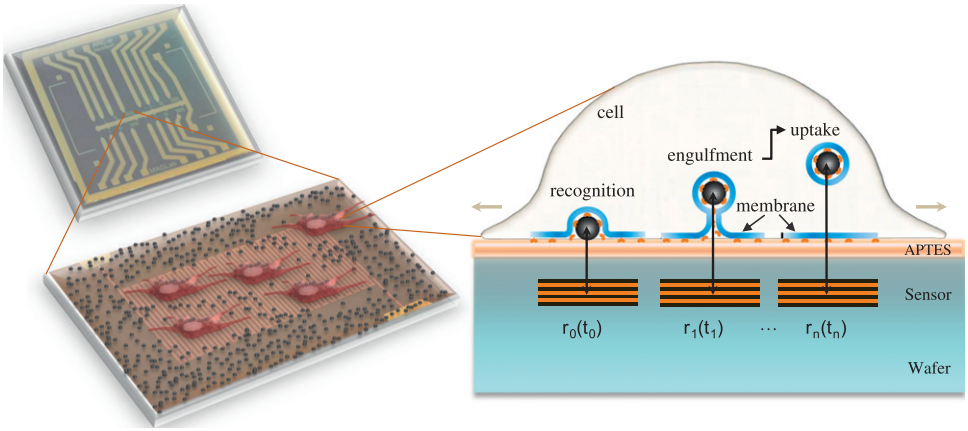

Fig. 1. (a) Sketch of spread cells on top of a magnetoresistive sensor surface covered by pre-immobilized magnetic particles. (b) Cross-section sketch of phagocytosis stages: After particle recognition, the cell starts engulfing and finally internalizing the particle in phagosomes, leading to an increase of the particle-to-sensor distance $r(t)$.

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