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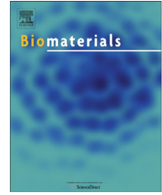

Cell guidance into quiescent state through chromatin remodeling induced by elastic modulus of substrate

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ABSTRACT

Substrate stiffness is known to strongly influence the fate of adhering cells. Yet, little is known about the influence of the substrate stiffness on chromatin. Chromatin integrates a multitude of biochemical signals interpreted by activation or gene silencing. Here we investigate for the first time the organization of chromatin of epithelial cells on substrate with various mechanical properties. On stiff substrates (100–200 kPa), where cells preferentially adhere, chromatin is mainly found in its euchromatin form. Decreasing the Young modulus to 50 kPa is correlated with a partial shift from euchromatin to heterochromatin. On very soft substrates ($\ll$10 kPa) this is accompanied by cell lysis. On these very soft substrates, histone deacetylase inhibition by adding a drug preserves acetylated histone and thus maintains the euchromatin form, thereby keeping intact the nuclear envelope as well as a residual intermediate filament network around the nucleus. This allows cells to survive in a non-adherent state without undergoing proliferation. When transfer on a stiff substrate these cells retain their capacity to adhere, to spread and to enter a novel mitotic cycle. A similar effect is observed on soft substrates (50 kPa) without need of histone deacetylase inhibition. These new results suggest that on soft substrates cells might enter in a quiescence state. Cell quiescence may thus be triggered by the Young modulus of a substrate, a major result for strategies focusing on the design of scaffold in tissue engineering.

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1. Introduction

Engineered materials are essential to develop soft tissue scaffolds used in regenerative therapies. However, strategies to enhance survival of cells within these scaffolds remain a significant challenge. Cells of multicellular organisms respond to biochemical and mechanical environmental signals in variable ways, realizing physical events like adhesion, migration, contraction and protrusion with great adaptability. To achieve these dynamic processes, extra- and intracellular forces are transmitted across the cytoskeleton to the nucleus. These forces can activate integrins at focal adhesions linked to actin filaments, themselves connected to microtubules and to intermediate filaments (IFs). The LINC complex (linker of nucleoskeleton and cytoskeleton), which enables force transmission across the nuclear envelope, connects cytoskeletal filaments to the nucleus where lamins form an extended part of the LINC complex [1–6]. These forces ultimately propagate to chromatin that represents a site of signal integration and interpretation for genes expression [7,8]. Further, recent works suggested that the nucleus itself may act as a cellular mechanosensor bypassing diffusion-based mechano-signaling through the cytoplasm [4,9]. Thereby, Swift et al. revealed that the nuclear lamina functions as a nuclear force sensor [10]. Importantly, mutations in nuclei-associated proteins result in a large number of diseases [11,12].

Two well-defined cytological compartments are considered in the nucleus: the condensed, inactive heterochromatin and the extended, active euchromatin. Heterochromatin is restricted to an irregular rim located at the nuclear periphery and around the nucleolus as well as in patches throughout the nucleoplasm,

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whereas euchromatin fills up the majority of the nucleus. The opposing actions of histone acetyl transferases and histone deacetylases (HDACs) dynamically control the acetylation status of chromatin and hence chromatin compaction [13,14], by respectively loosing (euchromatin) or condensing chromatin structures (heterochromatin). Chromatin organization has a strong influence on the expression of the genome [15] and chromatin remodeling contributes to many cellular properties as for instance cell pluripotency and cell differentiation [16], as well as to the deformation of the nucleus [17–21].

While mechanotransduction processes by which cells sense substrate stiffness was extensively studied on mechanosensitive proteins at focal adhesions and inside the cytoskeleton [22–25], little is known about how chromatin plasticity is influenced in response to changes of substrate elasticity. Our group previously studied the behavior of marsupial kidney epithelial (PtK2) cells deposited on polyelectrolyte multilayers (PEMs) [26,27] made of poly(L-lysine)/hyaluronic acid $(PLL/HA)_{24}$ stratum capped with a poly(sodium styrene sulfonate)/poly(allylamine hydrochloride) $(PSS/PAH)_n$ multilayer film as substrate models mimicking the extracellular matrices elasticity of biological tissues [28–30]. In this model, the rigidity of the film decreases by reducing the number n of PSS/PAH layer pairs (Fig. S1). We evidenced that soft substrates with a Young's modulus E of about 50 kPa prevented the formation of focal contacts and actin stress fibers and subsequently the activation of DNA replication whereas genes transcription was preserved. In contrast, rigid substrates with Young's modulus of 200 kPa allowed the formation of focal contacts and stress fibers necessary for DNA replication [29].

In the present manuscript, we investigate the impact of substrate elasticity on nuclear components, which led us to demonstrate that the remodeling between euchromatin and heterochromatin, together with the nuclear envelope connected to IF network, are major determinants of the response of epithelial cells to external mechanical signals.

2. Experimental

2.1. Notations

We shall use the short-hand notations E_0, E_{20} and E_{50} and E_{200} for the $(PLL/HA)_{24}$, $(PLL/HA)_{24}$–$(PSS/PAH)_n$ films with $n = 0, 1, 2$, and 5 respectively (see Fig. S1). E_0 and E_{20} are considered as very soft substrates, E_{50} is a soft substrate and E_{200} is considered as stiff substrate. The tiny letter in figures refers timing and conditions of the experiments schematized in Table 1.

2.2. Materials and fabrication of PEM

PLL (MW = 5.7×10^4 Da, Sigma, St. Quentin Fallavier, France) and HA (MW = 4.0×10^5 Da, BioIberica, Barcelona, Spain) were used for buildup $(PLL/HA)_{24}$ films, and PSS (MW = 7.0×10^4 Da, Sigma) and PAH (MW = 7.0×10^4 Da, Sigma) for $(PSS/PAH)_n$ capping films (n corresponds to the number of layer pairs), which were deposited on top of $(PLL/HA)_{24}$ strata. PLL, HA, PSS, and PAH were dissolved at 1 mg/mL in a buffer solution containing 150 mM NaCl and 20 mM of tris(hydroxymethyl)-aminomethane (TRIS) at pH 7.4, and all rinsing steps were performed in the same buffer. $(PLL/HA)_{24}$ strata and $(PSS/PAH)_n$ capping films were prepared using a dipping machine (Dipping Robot DR3, Riegler & Kirstein GmbH, Berlin, Germany), on glass slides (VWR Scientific, Fontenay sous Bois, France). The rigidity of the $(PLL/HA)_{24}$–$(PSS/PAH)_n$ film increases with the number of deposited PSS/PAH layer pairs (Fig. S1) [26].

2.3. Apoptotic, necrotic, healthy cells assay

Cells were seeded on surfaces at $1 \cdot 10^5$ per cm^2. Apoptotic, necrotic and healthy cells were carried out using the apoptotic/necrotic/healthy cells detection Kit (PromoKine) according to the manufacturer's instructions. Cells were observed by fluorescence microscopy (see Section 2.5) and analyzed by ImageJ (http://rsb.info.nih.gov/ij/). Briefly, using ImageJ, the 3 channels blue, green and red corresponding respectively to the labeled cells with Hoechst 33342 (blue only, DNA), Annexin V-FITC (apoptotics cells) and EthD-III (necrotic cells) were superimposed. Quantification of positive cells in these different signals was done using the ImageJ plugin "Cell Counter".

2.4. Cell culture

PtK2 cells (CCL-56, LGC Standards, United Kingdom) were grown in Eagle's minimum essential medium (EMEM, LGC Standards) supplemented with 100 μg mL^{-1} penicillin, 100 μg mL^{-1} streptomycin (Invitrogen, Carlsbad, CA), and 10% fetal bovine serum (Invitrogen) maintained at 37 °C with 5% CO_2. For histone deacetylase activity (HDAC), transcriptional activity and vimentin inhibitor treatments, cells were cultured respectively with 50 nM tricostatin A (TSA, Sigma), with

Table 1
Conditions and timing of the experiments.

Notation	Conditions and timing of the experiments	Notation	Conditions and timing of the experiments
glass-a	0 — 5 (h), glass	E_{20} + TSA-i	0 —//— 24 (h), glass → 0 +TSA 5 (h), E_{20}
E_{200}-b	0 — 5 (h), E_{200}	E_0 + TSA-j	0 —//— 24 (h), glass → 0 +TSA 5 (h), E_0
E_{50}-c	0 — 5 (h), E_{50}	E_0 + TSA-k	0 +TSA —//— 24 (h), glass → 0 +TSA —//— 24 (h), E_0
E_{20}-d	0 — 5 (h), E_{20}	glass + AMA-l	0 +AMA —//— 24 (h), glass → 0 +AMA 5 (h), glass
E_0-e	0 — 5 (h), E_0	E_0 + TSA + AMA-m	0 +TSA +AMA —//— 24 (h), glass → 0 +TSA +AMA 5 (h), E_0
E_{50}-f	0 —//— 24 (h), E_{50}	E_0 + TSA + IDPN-n	0 +TSA —//— 24 (h), glass → 0 +TSA +IDPN 5 (h), E_0
E_{20} + TSA-g	0 +TSA —//— 24 (h), glass → 0 +TSA 5 (h), E_{20}	glass + IDPN-o	0 +IDPN 5 (h), glass
E_0 + TSA-h	0 +TSA —//— 24 (h), glass → 0 +TSA 5 (h), E_0		

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