SKELETAL MUSCLE CELLS ADHESION AND DIFFERENTIATION ON POLYELECTROLYTE MULTILAYER FILMS OF CONTROLLED STIFFNESS

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In the field of biomaterials, controlling the surface properties of the materials is of crucial importance as these properties can influence cell behaviours. Beside chemical properties and topographical features, the role of the mechanical properties of the matrix has begun to be unravelled [1]. Matrix stiffness was first found to have an effect on cell morphology, adhesion, proliferation. More recently, a new focus has emerged since the rigidity of a matrix was also found to influence higher order cellular processes such as cell differentiation [2].

The layer-by-layer technique that allows preparing polyelectrolyte multilayer films (PEM) is a powerful tool to investigate interaction between matrix and cells. These surface coatings can be deposited easily on biomaterial surfaces and have versatile properties [3]. First, their bulk and surface chemistry can be modulated by carefully choosing the type of polymer used in the buildup and the film end-layer. Second, their "bulk" mechanical properties can be modulated, either by incorporation of various functional materials, or by covalently cross-linking the films. Using the carbodiimide chemistry, we recently prepared a wide range of multilayer films made of poly(L-lysine) and hyaluronic acid (PLL/HA) films of increased stiffness by simply varying the cross-linker concentration [4]. The film physical-chemical properties are gathered in table 1. The film Young's modulus exhibits the most important variation upon cross-linking as compared to wettability, roughness and serum adsorption.

Here, we used (PLL/HA) cross-linked films at various degrees to investigate skeletal muscle cell differentiation. Prior to differentiation, adhesion and proliferation of C2C12 cells on the films have been first investigated. On native (un-crosslinked) and soft films, cells can not adhere nor spread. Films with low crosslinking density (EDC <10) are not favourable for cell adhesion and proliferation, whereas highly cross-linked films showed a significantly increased adhesion and proliferation (Fig 1). Immunostaining for vinculin and F-actin revealed that stiff films promoted the formation of focal adhesions and the organization of the cytoskeleton.

C2C12 cells were then switched to a low serum containing medium for examining terminal cell differentiation into myotubes. Interestingly, although the expressions of myogenic differentiation markers like Troponin T, myogenin and myf5 are very similar for all film types, the morphology of the myotubes exhibited striking stiffness-dependent differences. Soft films allowed differentiation only for few days and the myotubes were very short and thick. Cell clumping followed by aggregates detachment was observed after ~2 to 4 days. On stiffer films, significantly more elongated and thinner myotubes were observed for up to ~ 2 weeks (Fig. 2, 3). In later stages of differentiation, myotubes striation was also observed but only for the stiffest films (EDC >50).

In conclusion, we demonstrate that film stiffness modulates deeply not only initial myoblast adhesion, proliferation but also myoblast differentiation into myotubes. Differences in cell motility, in cell/film or cell/cell molecular contacts may be the reasons for these observations. Further studies will be conducted to fully elucidate this point.

The present findings highlight the important role of surface mechanical properties in cell/material interactions. These PEM films could find applications in the fields of regenerative medicine and muscle/cardiac tissue engineering, where controlling the cell/material interactions is crucial for guiding the cellular response. On a more fundamental point of view, these nanoscale films are particularly relevant to cell studies, as many different parameters, and among which mechanical properties, can be varied. Future investigations of subcellular responses to mechanical cues are thus foreseen.

References:

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Table 1. Summary of the physico-chemical properties of cross-linked $(PLL/HA)_{12}$ films for EDC varied between 5 and 100 mg/mL (EDC*x* means that EDC final concentration is *x* mg/mL). Young's modulus was determined by AFM nano-indentation experiment, film roughness was determined from AFM images of film topography. Serum adsorption was measured by quartz crystal microbalance.

Type of films	Young's modulus E_0 (kPa)	Fold increase	Roughness (nm)	Serum adsorption (ng/ cm²)
Native	3	1	1.1 ± 0.3	1947
EDC5	99	~33	NA	NA
EDC10	162	~54	3.8 ± 0.5	88
EDC30	301	~100	5.8 ± 0.4	62
EDC50	354	~118	7.1 ± 1.4	44
EDC100	382	~127	7.4 ± 0.2	177

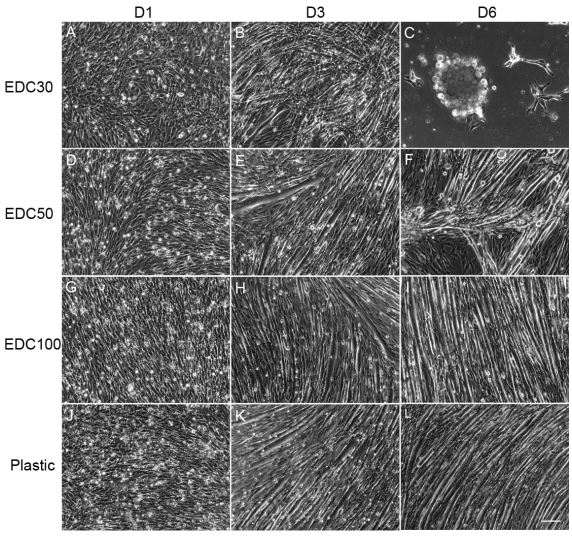

Figure 2. Phase contrast microscopy observations of C2C12 cells differentiation cultured in DM on $(PLL/HA)_{12}$ films cross-linked at various EDC concentrations at day 1 (left), day 3 (middle) and day 6 (right), respectively: EDC30, 300 kPa (A, B, C), EDC50, 354 kPa (D, E, F), EDC100, 382 kPa (G, H, I) and tissue culture polystyrene, P (J, K, L) (Scale bar is 100 µm).

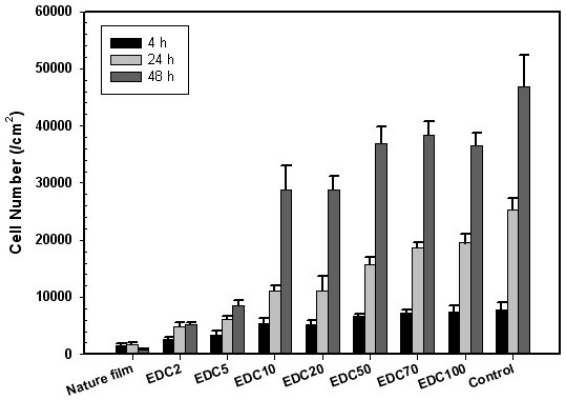

Figure 1. Cell adhesion and proliferation depend on film cross-linking. Quantification of the number of adherent cells on the cross-linked $(PLL/HA)_{12}$ films for increasing EDC concentrations, after 4h, 24h and 48h in growth medium. (data are means ± standard deviation, SD of three independent wells or slides).

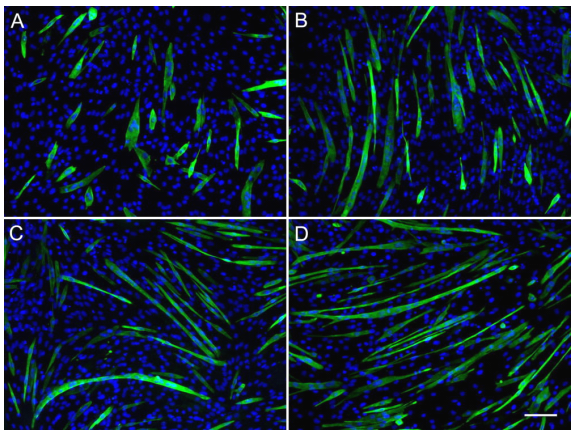

Figure 3. Immuno-fluorescence images of troponin T (green) and DNA (blue) of C2C12 cells cultured on $(PLL/HA)_{12}$ films cross-linked at various EDC concentration after 3 days in DM: EDC30 (A), EDC50 (B), EDC100 (C), as compared to tissue-culture plastic (D) (Scale bar is 100 µm).