Effect of microtopography and cell concentration on T lymphoid cell migration during in vitro extravasation process.

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ABSTRACT

The locomotion of leukocyte cells has been proved to be influenced by microtopographical patterns with and without surface chemistry. In this study, the migration of lymphoid cells was observed on microfabricated and flat substrates to investigate the incidence of microtopography on cell adhesion and locomotion response. Cell migration was investigated by two independent parameters. These were i) the cell type and ii) the cell population concentrations used over the same microtopographical patterns.
Thus, the objective of this work was to demonstrate that the migratory response of malignant T lymphoid cells (HUT78) was enhanced, compared to healthy T lymphoid cells (PBTL) by the topographical patterns of the substrate. These results were also independent from the initial cell adhesion and cell concentrations.
This difference may have exploitable applications for the investigation of the inflammatory response of migrating cells on artificially engineered substrates (i.e., dental or bone implant replacement).

Keywords: **T lymphoid cell, cell concentration, microtopography, cell migration, extravasation process.**

INTRODUCTION

The locomotion of leukocyte cells has been proved to be influenced by microtopographical patterns with and without surface chemistry [1, 2]. In this study, parallel grooves were microfabricated on borosilicate glass surface by wet etching process to create transparent substrates for time lapse microscopy analysis. The locomotory behavior of lymphoid cells was observed on the microfabricated substrates and compared with plasma treated flat substrates to investigate the incidence of microtopography on cell adhesion and locomotion response. For this purpose the geometrical features of the parallel microchannels (i.e., width, height and depth) were kept constant (Fig.1). Cell migration was investigated by two independent parameters. These were i) the cell lineage type and ii) the cell population concentrations used over the same microtopographical patterns. Peripheral blood T lymphocytes (PBTL) and malignant human T lymphoma cell line (HUT78) have been shown to have similar migratory behavior and cell signaling response during in vitro extravasation process [3]. The cell population concentrations used were mimicking i) single cell migration (low cell population 1.5 x 10^5 cells/ml) and ii) physiological concentration (high cell population 5 x 10^5 cells/ml).

Thus, the objective of this work was to demonstrate that the migratory response of malignant T lymphoid cells (HUT78) was enhanced, compared to healthy T lymphoid cells (PBTL) by the topographical patterns of the substrate.

Therefore, in this work it was found that the surface topography can influence the motile response of the two different T cell types in different ways, and this can be quantified in terms of motility parameters. This difference may have exploitable applications in static and dynamic fluidic systems for the investigation of the inflammatory response of migrating cells (i.e., cell sorting, cell separation).

METHODS

1.1 Microfabrication of parallel grooves on borosilicate glass substrate.

Borosilicate glass chips (1x1 cm) were patterned using a standard microlithography technique [2]. Patterns were designed using a specific CAD software (Kic, Whiteley Research Inc., USA) and transferred by shadow mask contact using a UV mask aligner (MJB3 UV 400, Karl Suss, Germany). After development of the photoresist the patterns were created by chemical selective etching of borosilicate microscope slide surfaces (VWR, USA). The chemical selective etchant used in this study was a commercially pure fluorosilicic acid (H_2SiO_6) (Fisher Scientific, UK). After that an Oxygen plasma treatment dose (Junior Plasma, Europlasma, Belgium) was given to the etched samples to homogenize the hydrophilic superficial properties of the glass chips. Then, the hydrophilicity of each surface was measured over seven days to check the decay time of the properties (FTA 125, FTA, USA). Once plasma treated, the glass chips were kept in 70% ethanol solution to reduce surface oxidation and contamination.

1.2 Microfabrication of parallel grooves: pattern geometry selection.

The topographical geometry used in this study was an equally spaced array of lines. This pattern was chosen because it is approximating the structure of the endothelial wall, and also because widely used in literature [4]. The geometrical dimensions of the topographical pattern were determined by a direct measure of the cell types under investigation. In fact, the idea behind is that any difference in cellular morphology can be used as a physical barrier to trigger different cell behavior response. The groove geometrical dimensions (i.e., width, and height) were determined by directly measuring the cell size under investigation. It was found that HUT78 has a diameter of 12.34 ± 2.88 μm whereas PBTL has a diameter of 7.31 ± 1.25 μm (n=300 cells approx.) [5]. This difference in cell dimension suggested that a 10 μm space between grooves could be used as a physical barrier to which each cell types would respond differently.

The final product was a $1cm^2$ transparent borosilicate glass chip with a series of parallel channels with a U profile (typical of the etching technique) with a width of w = 10 μm (bottom of the channel) and depth d = 3.5 μm. The cross-section geometry and surface nano-roughness were accurately measured along the full length of the pattern (l = 200 μm), as extensively reported in [2].

1.3 Characterization of micropatterned surfaces.

The micropatterned chips were fully characterized in 3D by White Light Scanning Interferometer (WLSI) (New View 100, Zygo Corporation, USA) technique. Then, each groove cross-sections were also precisely checked by WLSI technique in a considerable short length of time. The main advantages of WLSI measuring technique are that it does not require any specific surface treatment and it is not an invasive or destructive.

1.4 T Lymphoid cell culture.

Two types of human T cells were used in our experiments as previously described (Prina_Mello et al., 2003). In brief two T lymphoid cells were used in this study (i) normal human peripheral blood T lymphocytes (PBTLs) and (ii) malignant T lymphoma cell line HUT78 (HUT78s) (ATCC, USA). While PBTLs were pre-activated with phorbol myristate acetate (PMA) (Sigma, USA) and incubated at 37°C, 5% CO_2 for 48 hours, the HUT78s were only incubated for 60 minutes [3]. Both cell types were cultured in RPMI 1640 supplemented with HEPES buffer (Life Technologies, U.K.), antibiotics and 10% fetal bovine serum (FBS) at a concentration of approximately 6 x 10^5 cells/ml.

1.5 Cell population concentration on microfabricated surfaces.

In this study, for both cell types (i.e., HUT78 and PBTL) identical cell concentrations were used to investigate i) single cell migration and ii) physiological concentration. For single cell migration a low cell concentration of 1.5 x 10^5 cells/ml was used to have an average cell population distribution of 10 x 10^3 cell/well. For the physiological migration a high cell concentration of 5 x 10^5 cells/ml was used to have an average cell population distribution of 35 x 10^3 cell/well.

1.6 T lymphoid cell adhesion to micropatterned surfaces

Cell activation on a glass substrate was obtained after coating the substrate with monoclonal antibodies (mAb) (LFA-1, Sanbio, The Netherlands). After 48 hours of culture of HUT78 or activated PBTL, at the above specified concentrations, were placed on top of the mAb coated borosilicate substrates [3]. The substrates were then covered with a 1.5 ml of medium solution. Control chambers were treated similarly with IgG (Dako, Bucks, UK). Cells were incubated for one hour in culture medium to stimulate adhesion and polarization. Unattached cells were removed by triple gentle washing of wells with warmed culture medium. It was observed that LFA-1 induced cytoskeletal changes and consequent adhesion in T cells, as shown in Prina_Mello *et al*. [2].

1.7 Cell migration on microfabricated surfaces

Cell migration and response to different concentration was tracked by time lapse recording using an inverted microscope (Nikon Eclipse TE-300, Japan) with CCD camera (Leica DC 100, Germany) to observe and analyze cell motion within each field selected. A minimum of three fields were analyzed from each specimen (each field contained at least 10 cells). Temperature and humidity were controlled during tracking (temperature 37 ± 0.1°C, humidity <40%). The details of this method have been published previously [2].

In brief, over 60 minutes observation individual and group of cells were tracked on their cellular recombinant changes.

Over the full length of time cell movement was monitored by a semi-automated image analysis system (Scion Image, Scion Corp., NIH, USA). Digital images of the field of view were collected every five minutes. From each image an extended set of information was measured and calculated to investigate adhesion, polarization and migration. This set was constituted by the perimeter, the enclosed area of the cell, the direction angle, and the cell centroid (x and y position of the center of a two-dimensional non-regular geometrical shape).

From the cell centroid the squared displacement of around 30 cells was calculated for every time interval. Two different equations have been used to calculate the mean square displacement of the migrating cells when moving on the two topographical patterns.

The mean of the squared displacement as a function of time is described for a one-dimensional random walk by the following equation:

$$< T^2 >_t = 2 \cdot (S^2 P) \cdot (t - P + Pe^{-t/P}) \qquad (1)$$

where P is the persistence, t is the interval time and S is the cell speed calculated for each cell tracked. This is simplified to the following equation when the time is sufficiently large

$$< T^2 > = 2D^* \cdot (t - P^*) \qquad (2)$$

where D* and P* are parameters derived from the experimental data.

In the case of a two-dimensional random walk the mean of the squared displacement can be simplified to the following equation:

$$< T^2 > = 4D^* \cdot (t - P^*) \qquad (3)$$

The value of D^* and P^* in both cases were estimated from the slope of the intercept of the line, as described in previous works [2, 6, 7].

Those simplified equations were unconditionally used for both cell concentrations on the two topographical surfaces investigated.

RESULTS AND DISCUSSIONS

In this work several results were achieved. Firstly, regular microchannels were successfully fabricated on borosilicate glass by wet etching process. By simply controlling the etching time and the thickness of the photoresist it was possible to selectively determine the depth of the trenches in comparison with the overall etching depth, as shown in Fig. 1. The correlation between total depth and the groove depth was then measured by WLSI technique after the fabrication process (Fig. 1).

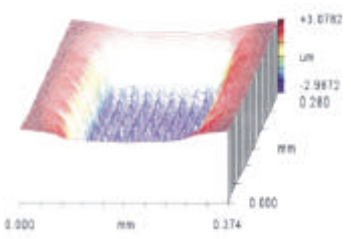

Figure 1

Material: Borosilicate glass
Wet etching: Hexafluorosilicic acid
Etch time: 10 minutes
Groove
sidewall: 5.9 μm
ridges: 1.15 μm
Roughness (Ra): 0.21 μm

Figure 1. Microtopography used in this study. Regular grid of microchannels with controlled nano-roughness.

The main objective of this work was to demonstrate that the migratory response of malignant T lymphoid cells (HUT78) was enhanced, compared to healthy T lymphoid cells (PBTL) by the topographical patterns of the substrate. These results were also independent from the initial cell adhesion and cell concentrations (Table 1).

Topography	*Cell type*	*Low cell conc.*	*High cell conc.*
Flat	**HUT78**	2.15 ± 0.73	1.43 ± 0.26
	PBTL	2.55 ± 0.67	1.65 ± 0.34
Microgrooved	**HUT78**	2.07 ± 0.50	1.27 ± 0.32
	PBTL	1.88 ± 0.61	1.40 ± 0.38

Table 1 Cell deformation index calculated to quantify cell adhesion at t_0 for HUT78 and PBTL cells under the two topographical conditions (mean and standard deviation).

In fact, for both concentrations it was found that, on the grooved pattern, the lymphoma HUT78 cells were more diffusive in their migration than the T lymphocyte PBTL cells (Fig. 2b, 3b). Whereas, on the flat surface, the T lymphocyte PBTL cells had a more diffusive response than the T lymphoma HUT78 cells (Fig. 2a, 3a). The enhanced migratory response of the HUT78 might have been triggered by the microtopography (i.e., nano/micro roughness). This microtopography activated cell migratory mechanism has been also confirmed in several other works for different cell lines (i.e., neutrophils, EC, fibroblasts) [7, 8, 9]. Here it was observed that the T lymphoid cells are undergoing the same cell migratory behavior for different cell concentrations. This directly implies that surface topography is the predominant extracellular stimuli to evoke cell migration with a consequent reduction in the cell-cell collision, normally present at high cell concentration.

In this work it was also found that malignant T lymphoma cells are significantly slower and less diffusive, for both cell concentrations, when exposed to a plane substrate than when subjected to a grooved substrate. This coincide with a reduced cytoskeletal cell activity (i.e., lamellipodia and filament re-orientation) mainly associated with the absence of external stimuli (i.e., chemical or physical) [10].

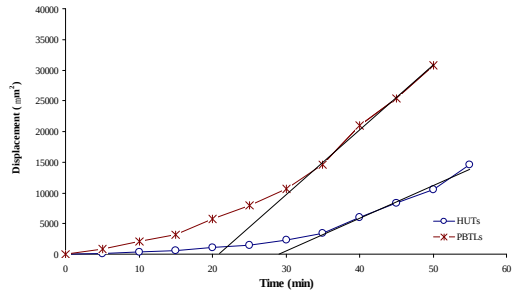

Figure 2a. Mean square displacement <T2> plotted against time for HUT78 and PBTL cells at low cell concentration over flat substrate (error bar = negligible).

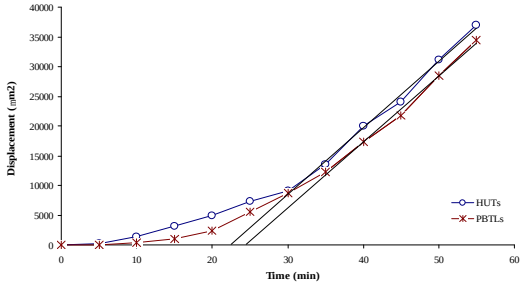

Figure 2b. $<T^2>$ plotted against time for HUT78 and PBTL at low cell concentration over *microgrooved substrate* (error bar = negligible).

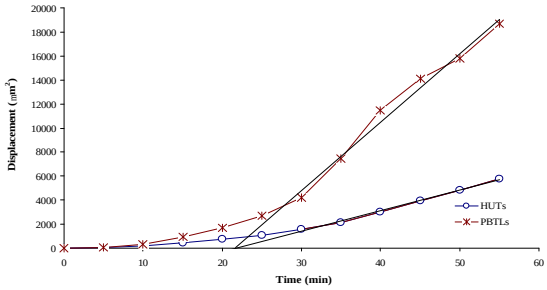

Figure 3a. $<T^2>$ plotted against time for HUT78 and PBTL at high cell concentration over *flat substrate* (error bar = negligible).

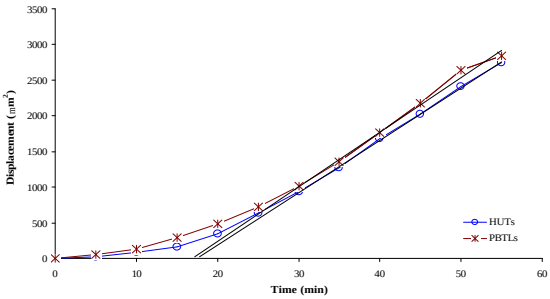

Figure 3b. $<T^2>$ plotted against time for HUT78 and PBTL at high cell concentration over *microgrooved substrate* (error bar = negligible).

CONCLUSIONS

This work highlights how surface topography can influence the motile response of the two different T cell types in different ways when activated in their migratory response over microfabricated substrates. This locomotory response can be quantified in terms of motility parameters (i.e., mean square displacement).

Therefore, the main results of this work can lead to a possible cell sorting system capable of high throughput T lymphoid cell separation based on micromanufactured surfaces.

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