

Investigation of Pulse electric field effect on HeLa cells alignment properties on extracellular matrix protein patterned surface

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Abstract

Cell behavior in terms of adhesion, orientation and guidance, on extracellular matrix (ECM) molecules including collagen, fibronectin and laminin can be examined using micro contact printing (MCP). These cell adhesion proteins can direct cellular adhesion, migration, differentiation and network formation *in-vitro*. This study investigates the effect of micro-contact printed ECM protein, namely fibronectin, on alignment and morphology of HeLa cells cultured *in-vitro*. Fibronectin was stamped on plain glass cover slips to create patterns of 25 μ m, 50 μ m and 100 μ m width. However, HeLa cells seeded on 50 μ m induced the best alignment on fibronectin pattern (7.66° \pm 1.55SD). As a consequence of this, 50 μ m wide fibronectin pattern was used to see how fibronectin induced cell guidance of HeLa cells was influenced by 100 μ s and single pulse electric fields (PEF) of 1kV/cm. The results indicates that cells aligned more under pulse electric field exposure (2.33° \pm 1.52SD) on fibronectin pattern substrate. Thus, PEF usage on biological cells would appear to enhance cell surface attachment and cell guidance. Understanding this further may have applications in enhancing tissue graft generation and potentially wound repair.

Keywords: HeLa; cells alignment; pulse electric field; micro contact printing; extra cellular matrix, cell proliferation, cell guidance

1. Introduction

Micro-contact printing (MCP) is a procedure that allows a surface to be well-designed with a material like extra cellular matrix (ECM) protein, in a clear outline [1-4]. Cell behavior in terms of adhesion,



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orientation and guidance, on ECM molecules such as fibronectin, collagen and laminin can be examined using MCP [5-6]. These cell adhesion proteins can guide cellular migration, adhesion and network establishment *in-vitro* [7]. These cellular characteristics can be used to deduce the cell signaling paths related with the cell interactions with the surface [8-9]. Fibronectin attach to collagen, fibroblasts in the ECM and the plasma membrane of the cells and its high molecular weight protein. This protein plays a vital role in cell adhesion and re-organisation of the ECM. The association of the cells and ECM is also facilitated by fibronectin. Fibronectin is a dimeric glycoprotein, consist of dimmers, with each around 250,000 molecular weight. Each of the subunits is doubled over three time (FN1, FN2, FN3) and contain the amino acid repeat FN1, FN2, FN3. Fibronectin can attach to collagen at FN1/2 region, whereas the FN3 region takes up contact with the respective cells [10, 7]. Alternative splicing forms completely different kinds of fibronectin. For example the liver builds fibronectin that flows as a liquefied element of the serum. Nevertheless, static fibronectin is also formed in various tissues [7].

In natural skeletal tissues, the cells and the structures form spatial and oriented geometries and patterns [11]. In recent research, significant work has been done to know cell surface attachment and alignment for biomedical applications [12-13]. The prospective uses of such biomedical systems consist of the micro-textures that helps in enhancing wound closure and tissue integration [14-15], [3-4]. Meanwhile, various polymer and metals have been commercially introduced. These metals and polymers frequently show poor attachment with the proteins and cells. This makes them hard to incorporate into the body tissues. To increase the bioactivity and biocompatibility, much work have been done to deposit bioactive coatings onto implant surfaces. Contact guidance can be promoted by the use of chemical guidance cues [16]. This is a fact which take account of the cell guidance, as they extend through the micro-patterns. Another researcher [17] observed the consequences of micro-patterned on cell guidance. In microfluidic devices, BioMEMS and lab-on-a-chip applications, Polydimethylsiloxane (PDMS) has been widely used [18-20] largely because of its biocompatibility and mechanical properties [21-22]. Researchers have also analyzed the consequences of micro-patterning on cell dispersion and guidance on PDMS [23, 9]. Formation of detailed surface designs on glass, metal or polymer might allow further clarification about the elements that control cell attachment, cell growth, variation and molecular signaling paths on material surfaces [24-25]. These surface-engineered materials are expected to be able to control the shape, size cell/cell coupling of the attached cells during guided network formation.

The aims of this study are to characterize HeLa cell guidance on micro contact printed fibronectin patterns and to then determine how that guidance is modified by pulse electric field.

2. Materials and methods

2.1. Cell culture

In this study, HeLa cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a 5% humidified CO₂ incubator. When the HeLa cells reached 80-90% confluence, media was removed from the tissue culture flask, then, 3ml of phosphate buffered saline (PBS) was added in the flask to wash the cells, which was removed after few seconds. 2 ml of TryPle Express from GIBCO was added to detach the cells from bottom of the flask. TryPle Express works best in a warm surrounding, so the flask was incubated for seven minutes at 37°C. After detachment, the HeLa cells were counted by haemocytometer. Thereafter, 800µl of cells suspension at a concentration of 3.75×10^4 cell/ml (equivalent to 30,000 cells) were transferred into a 4mm cuvette for electroporation.

2.2. Stamp fabrication

Main templates were made up of 25µm, 50µm and 100µm wide ridge (width) with 5µm deep channels at the University of Glasgow [26]. PDMS stamps were formed at the University of Bradford from

these templates. Initially, the template was coated by means of 2% dimethylchlorosilane mixed with 98% trichloroethylene which make the surface hydrophobic.

After that, 9ml of sylgard (silicon-based elastomer gel) and 1ml of curing agent were mixed with the templates and left to cure overnight. Vacuum processing was done to eliminate any air bubbles. After curing, the sylgard gel was removed from the template which enabled the generation of sylgard stamps showing a negative relief of the template to be formed. These stamps were then used in functionalizing plain glass slides and gold coated test substrates with various proteins. Figure 1 show the images of the PDMS stamps for 25 μ m, 50 μ m and 100 μ m width.

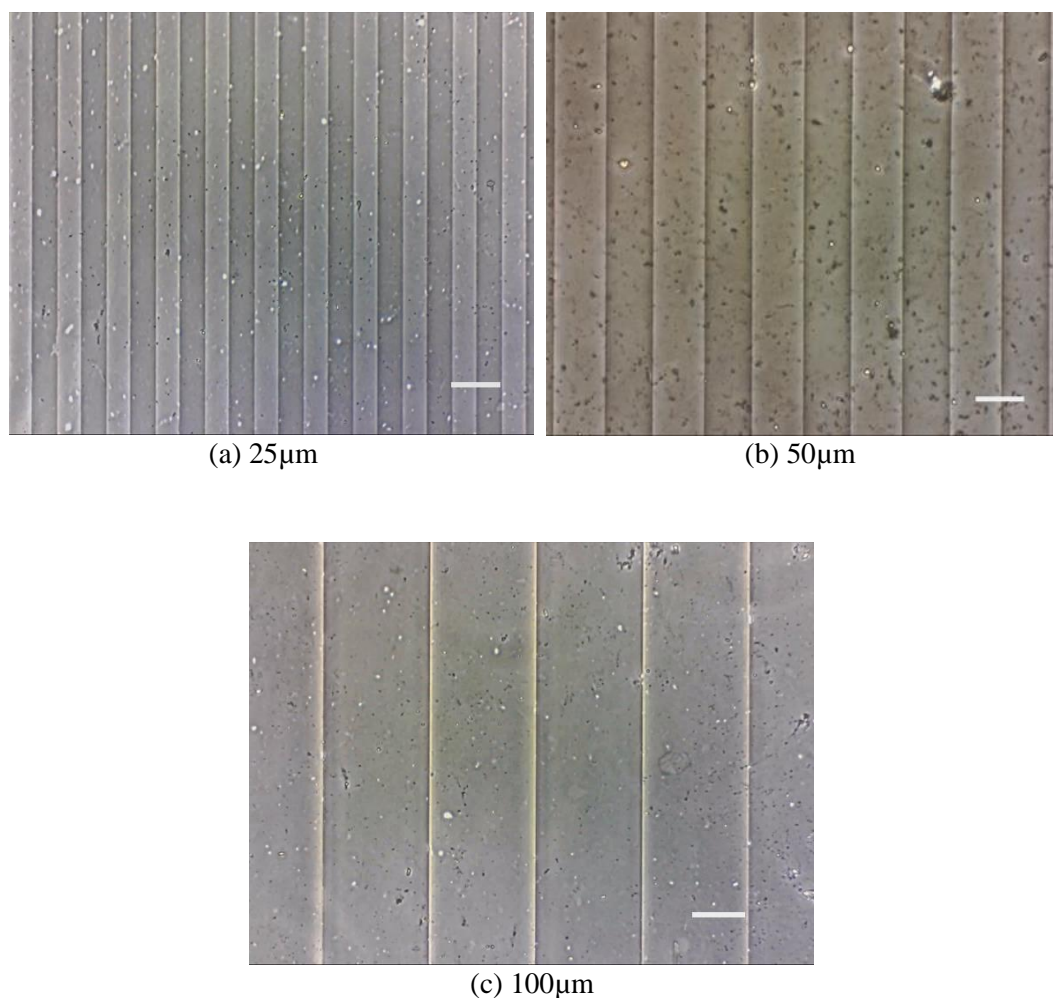


Figure 1. PDMS stamps with (a) 25 μ m, (b) 50 μ m and (c) 100 μ m width (scale bar 50 μ m)

2.3. Micro contact printing (MCP) technique

All working environments (surfaces) before MCP, stamps and coverslips were sterilized with 70% ethanol and air dried in a laminar flow hood. After that plain glass coverslips were micro-contact printed with fibronectin. Inking the PDMS stamps was achieved by applying a 50 μ g/ml solution of fibronectin in PBS [27] to the PDMS stamps for 60 seconds [28, 4]. Following inking the stamps were air-dried for 30 seconds. The stamps were then placed immediately in contact with the substrate and pressed slightly using another glass cover slip for 50 seconds [29]. The same method was repeated for

each stamp of the 25 μm , 50 μm and 100 μm stamps. This allowed the substrate to be patterned with 25 μm , 50 μm and 100 μm wide protein coated tracks which are separated by 25 μm , 50 μm and 100 μm wide uncoated tracks. Figure 2 demonstrates this process, in which the MCP patterns were successfully transferred to the substrates. Though imperfections in the stamp and the use of manual pressure caused some differences in the track widths of the stamped protein.

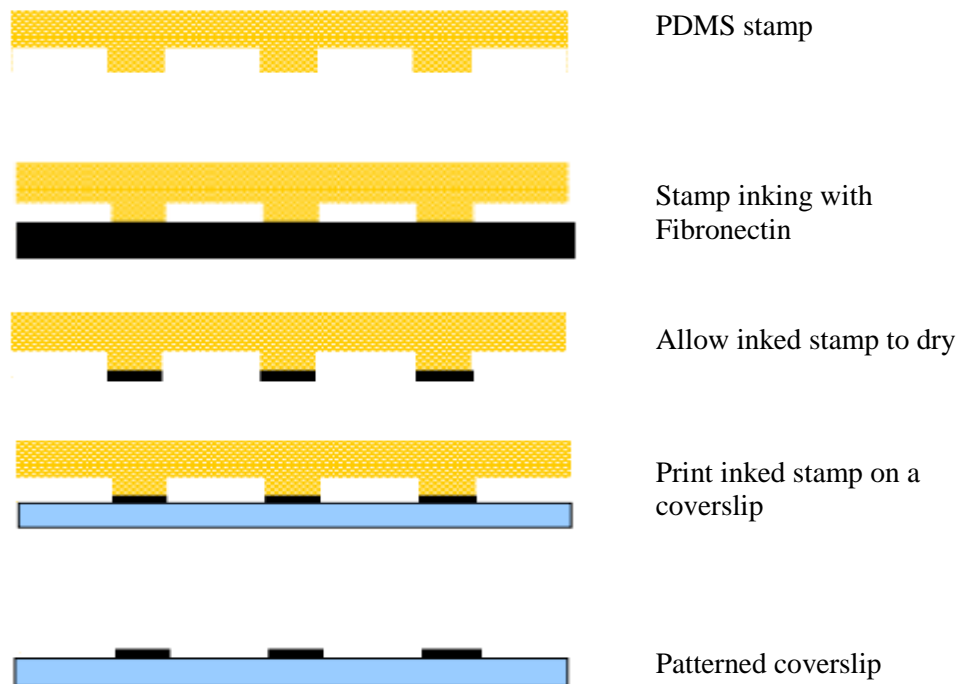


Figure 2. Illustration of the stamping process

2.4. Cell Plating

Plain glass slides and glass slides coated with un-patterned fibronectin were seeded with non-electroporated (NEP) HeLa cells at a concentration of 30,000 cells/coverslip for 18hrs as a control group. At the same time, glass slides with fibronectin micro-contact patterned printed with three different gratings (25 μm , 50 μm and 100 μm widths) were also seeded with non-electroporated HeLa cells for 18hrs. After 18hrs, cells were then imaged with standard phase contrast light microscope. The pattern with better alignment was used for seeding electrically treated HeLa cells. This allowed the impact of single pulse electric fields on cell guidance to be determined by comparing cell guidance on the optimum fibronectin pattern with and without PEF treatment.

3. Results and discussion

3.1. Micro contact printing (MCP) technique

The stamp consisted of ridges with 25 μm , 50 μm and 100 μm width fibronectin coated tracks separated by 25 μm , 50 μm and 100 μm width uncoated tracks. Micro-contact printing patterned of fibronectin on the cover glass (substrates) resulted in the substrates being successfully patterned with 25 μm , 50 μm and 100 μm width of repeated gratings as shown in Figure 3. The distances between the coated and uncoated channels have been marked by the yellow arrows.

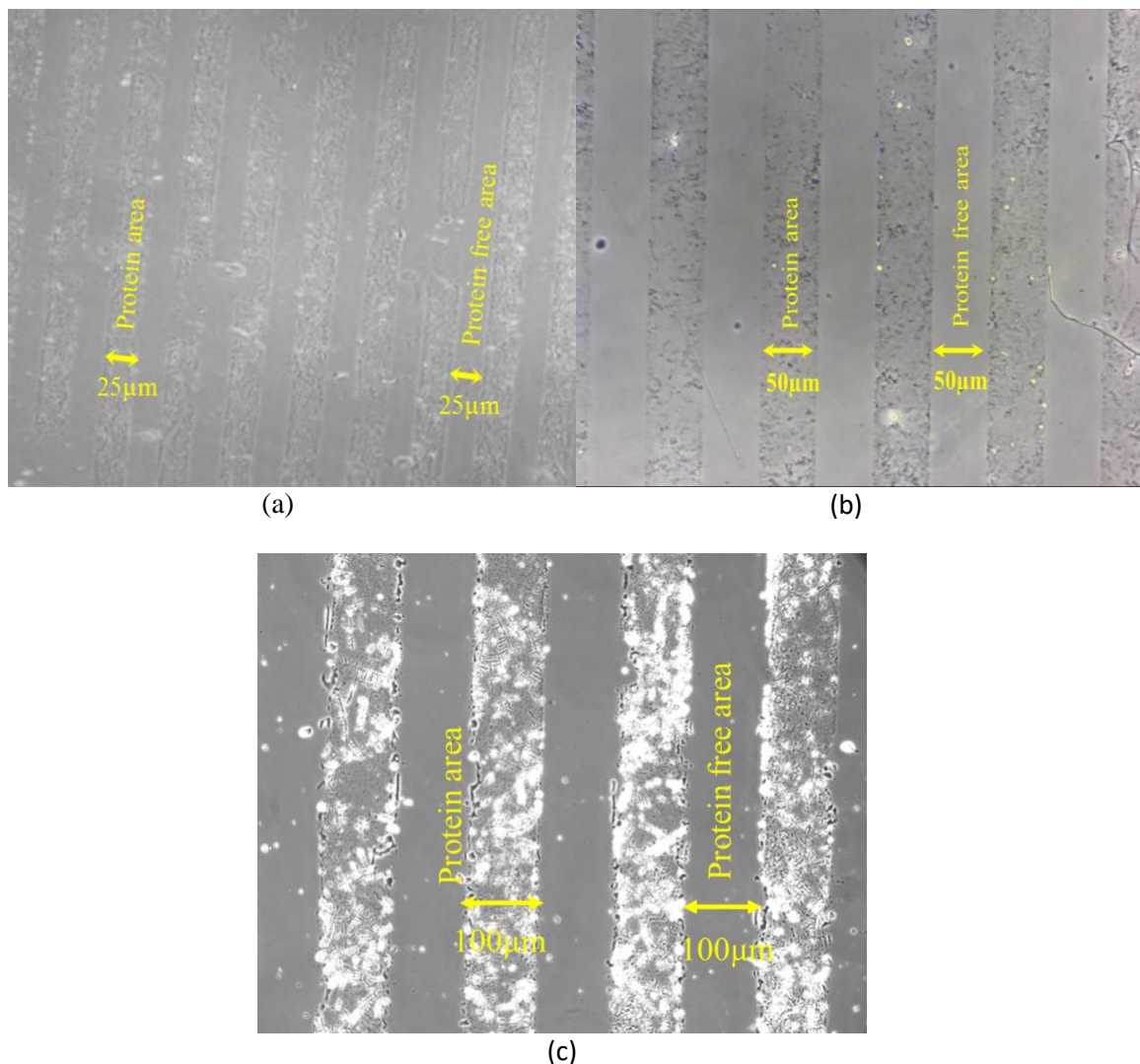
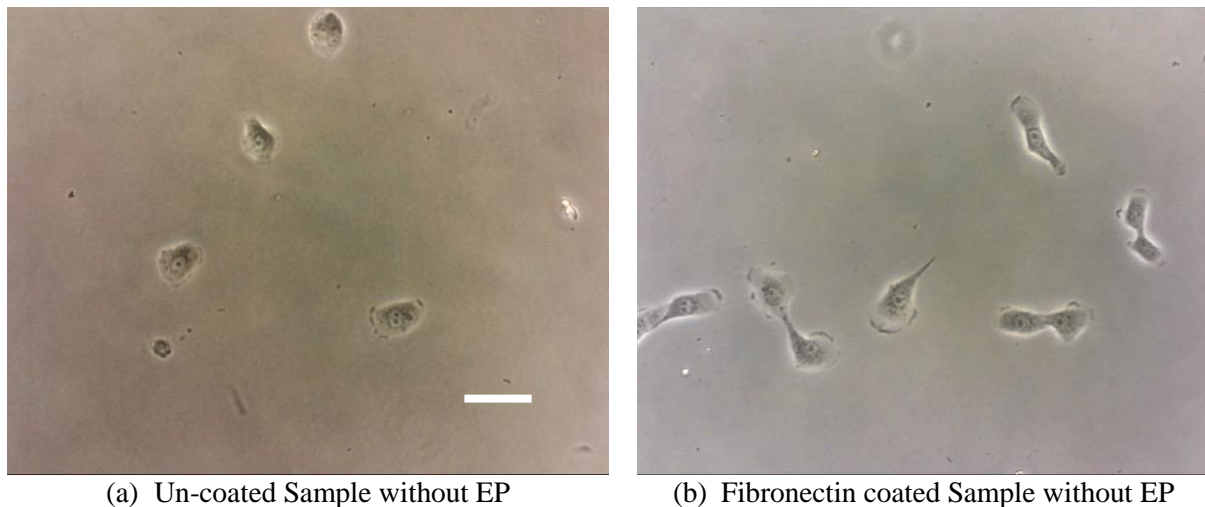


Figure 3. Actual images of (a) 25 μm , (b) 50 μm and (c) 100 μm width protein patterns printed on glass coverslip

3.2. *HeLa cells interactions with micro-patterned surface*

Substrate printed with chemical cues caused different cell responses like cell migration and cell alignment along the cues [1, 4, 17]. The effects of initial cell attachment on substrate were investigated by recording cell images up to 18hrs after cell culture using DinoCapture imaging software. After 18hrs in culture, the photomicrograph obtained from the plated HeLa cells showed different alignment to the MCP. It became clear that the HeLa cell seeded on uncoated and coated unpattern substrates (control group) did not show any alignment. This is obvious because even though coated but there is no pattern for the cell to follow. Figure 4 shows the morphology of HeLa cells on the two control group substrates (uncoated and fibronectin-coated but unpattern substrates). Even though the control group are identical in terms of initial cell seeding density, passage number and incubator environment, they showed different morphology and adhesion capacity when compared the pattern counterpart group. Most cell in the control group are rounded or rather more of an epithelial morphology which imply less adherence to the substrate [30].



(a) Un-coated Sample without EP

(b) Fibronectin coated Sample without EP

Figure 4. Photomicrographs of HeLa cells after 18hrs of seeding on (a) Un-coated coverslip glass, (b) Fibronectin coated on coverslip glass (control experiments) (scale bar 50 μ m)

However, the HeLa cells reacted differently to the various gratings of fibronectin patterned. HeLa cells seeded on 25 μ m patterned substrates induced a poor alignment ($34^\circ \pm 6.78SD$). On the other hand, 50 μ m induced best alignment of the cells to the fibronectin pattern ($7.66^\circ \pm 1.55SD$). Similarly, 100 μ m show good alignment ($12.06^\circ \pm 1.55SD$) as compared to the 25 μ m pattern but less than the 50 μ m pattern. As it shown in Figure 5, we can deduce that the fibronectin pattern substrate showed a better attachment with cells elongating along the pattern especially with the 50 μ m and 100 μ m though more articulate with 50 μ m.

Furthermore, the 50 μ m induced a better alignment was used to check the behavior of HeLa cells under the influence of electric field. Hence the cells were electroporated (with 1kv/cm 100 μ s & single pulse) and seeded on to a 50 μ m fibronectin micro-patterned substrate and their morphology compared to the non-electroporated counterpart. The results as shown in Figure 6 indicates that cells aligned ($2.33^\circ \pm 1.52SD$) on fibronectin pattern substrate especially under pulse electric field exposure: with better alignment of the cells in the EP group ($2.33^\circ \pm 1.52SD$), when compared to NEP group ($7.66^\circ \pm 1.55SD$) on the same 50 μ m fibronectin patterned substrate.

This could be that the electric field stimulated the signaling path way for cell adhesion and hence increase the cell spreading [31]. This finding is also in agreement to that of Zhang *et al.* (2011) [32]. Hence, in this research, it was also speculated that the pulse electric field could have stimulated the signaling path way, there by modulating the filopodia to align to the pattern with more pronounce elongation than the non-electrically treated counterpart. Thus, the outcome of the research can be utilized in cell assembling and guidance of cells for wound healing application and tissue regeneration for implantation, for example antibody fluorescence based experiments to measure specific integrin up-regulation.

Finally, the overall conclusion of this study is that PEF seems to enhance cell guidance on glass substrate micro-patterned with 50 μ m wide repeat gratings of fibronectin. This could indicate that in some way PEF and electroporation either modifies the distribution of fibronectin specific membrane bound integrin or results in their up, or down regulation. The manipulation of both may have implications in the enhancement of engineered tissue grafts technologies.

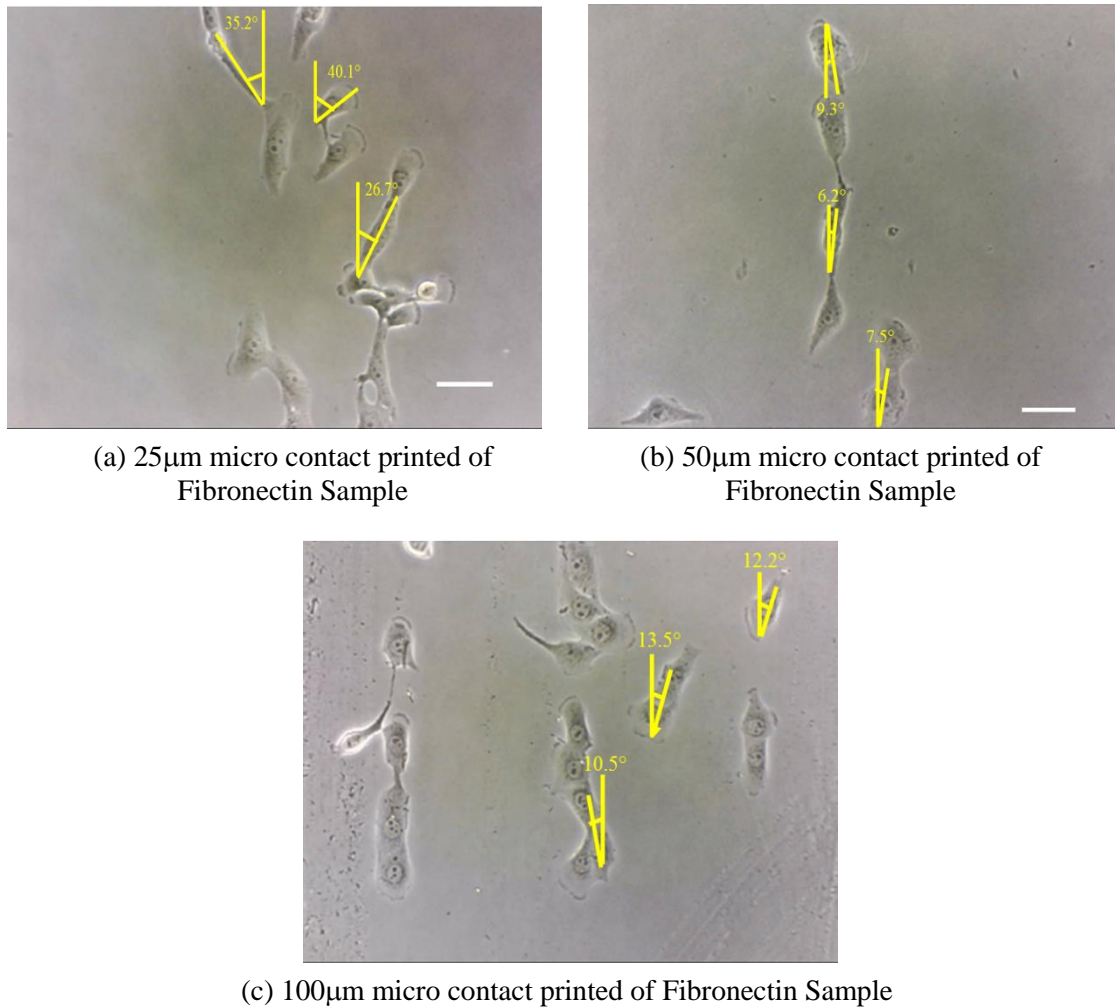


Figure 5. Photomicrographs of HeLa cells after 18hrs of seeding (a) 25µm, (b) 50µm and (c) 100µm micro contact printed of Fibronectin on coverslip glass (scale bar 50µm)

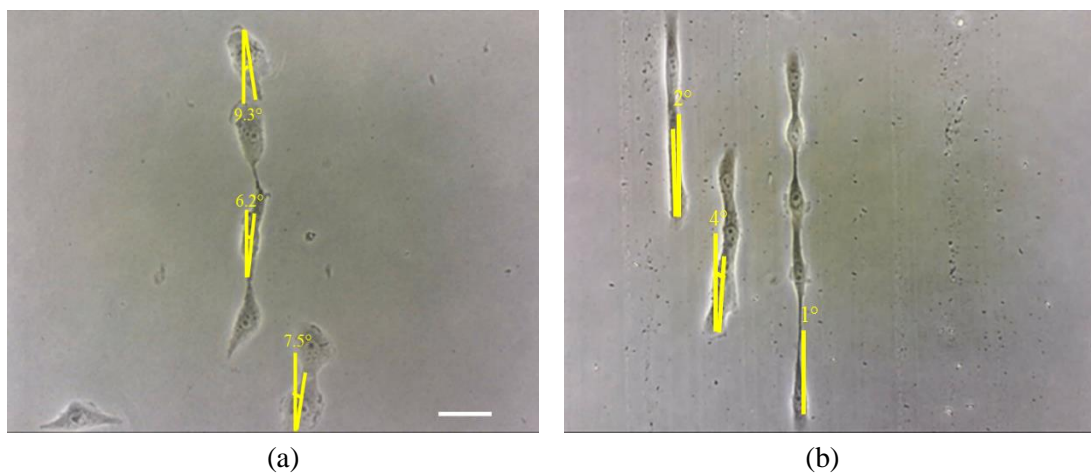


Figure 6. Photomicrographs of HeLa cells after 18hrs of seeding 50µm micro-contact printed of Fibronectin on coverslip glass (a) without EP and (b) with EP (scale bar 50µm)

4. Conclusion

This study mainly focuses on effects of PEC exposure on fibronectin pattern surface with HeLa cells for cell guidance. Stamp fabrication is performed in the first step of this experimentation. Once achieved, micro contact printing technique is achieved. This permitted the substrate to be patterned by 25 μ m, 50 μ m and 100 μ m width protein coated tracks. It was analyzed through experimentation that 50 μ m shows the most suitable for HeLa cells plating. This sample was also analyzed with PEF exposure. Results show that HeLa cells exposed to PEF show more aligned on the pattern surface when compared to control sample.

5. Acknowledgments

The researchers would like to thank Ministry of Higher Education Malaysia and UTHM for the research grant under the Tier 1 Research Grant (U865) for supporting this research work.

6. References

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